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ORIGINAL ARTICLES

CYTO-GENETIC ANALYSIS OF *SACCHARUM SPONTANEUM* L.

1.—CHROMOSOME STUDIES IN SOME INDIAN FORMS

BY

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(Received for publication on 15th August 1935)

(With Plates I-V)

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I. Introduction

The use of *Saccharum spontaneum* L. in sugarcane breeding has been fully justified by the results obtained both in India and Java. Beginning with Co. 205, which is an inter-specific hybrid between the thick cane " Vellai " and the local form of *S. spontaneum*, most of the canes produced at Coimbatore have in them traces of *spontaneum* ' blood '.

S. spontaneum is widely distributed in the Old World. It extends from Africa through Southern Asia to Australia and is represented in India by a number of distinct forms. Hackel classified the Indian varieties into,

Sub-species (*a*) *Indicum* with very narrow lamina and slender racemes ;
Sub-species (*b*) *Oegyptiacum* with broader lamina and dense racemes.

He mentions the prevalence of " intermediates " between these two sub-species. Hole [1911], while recognizing these different forms, considers them as purely ecological types.

The method of approaching taxonomic problems has changed considerably within the last decade and additional evidence of cytology and genetics is now brought to bear on the purely morphological studies on which species were hitherto differentiated. Our concept of species has, therefore, been very much widened.

In the present study an attempt is made to bring together observations on the chromosome behaviour of some of the Indian forms of *S. spontaneum* in the hope that the cytological facts presented will help to throw light on the phylogeny of this widely-distributed Linnean species.

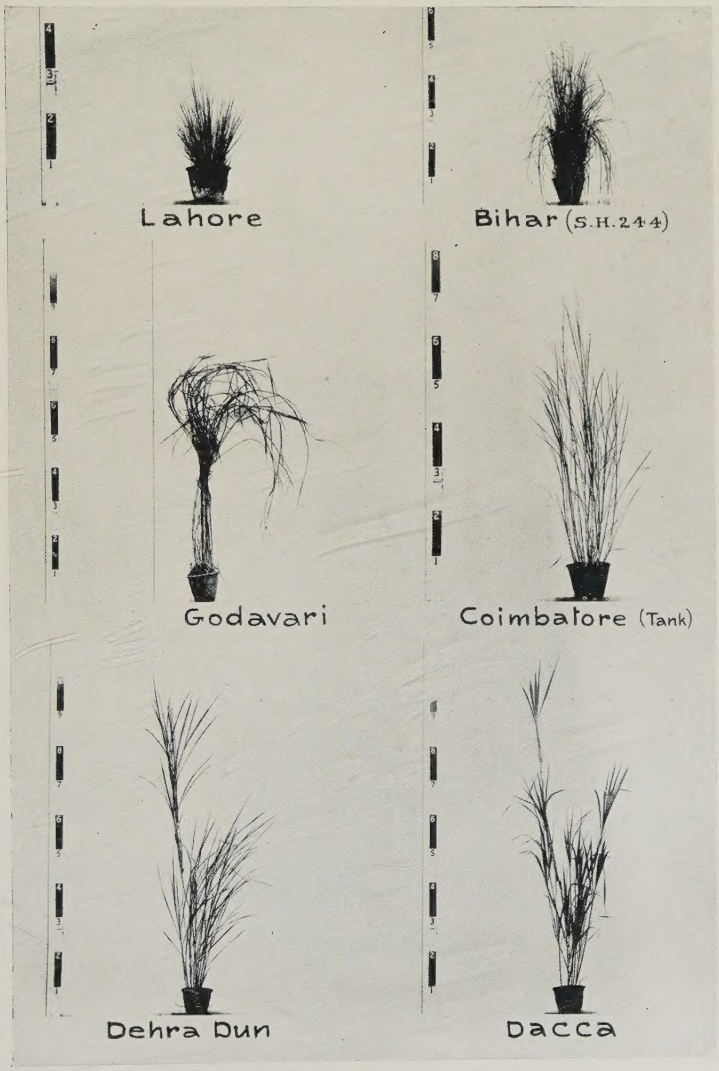
II. Material and methods

The material studied was grown at the Imperial Sugarcane Breeding Station, Coimbatore. The different types are named after the places from which they were collected. The following types were examined :—

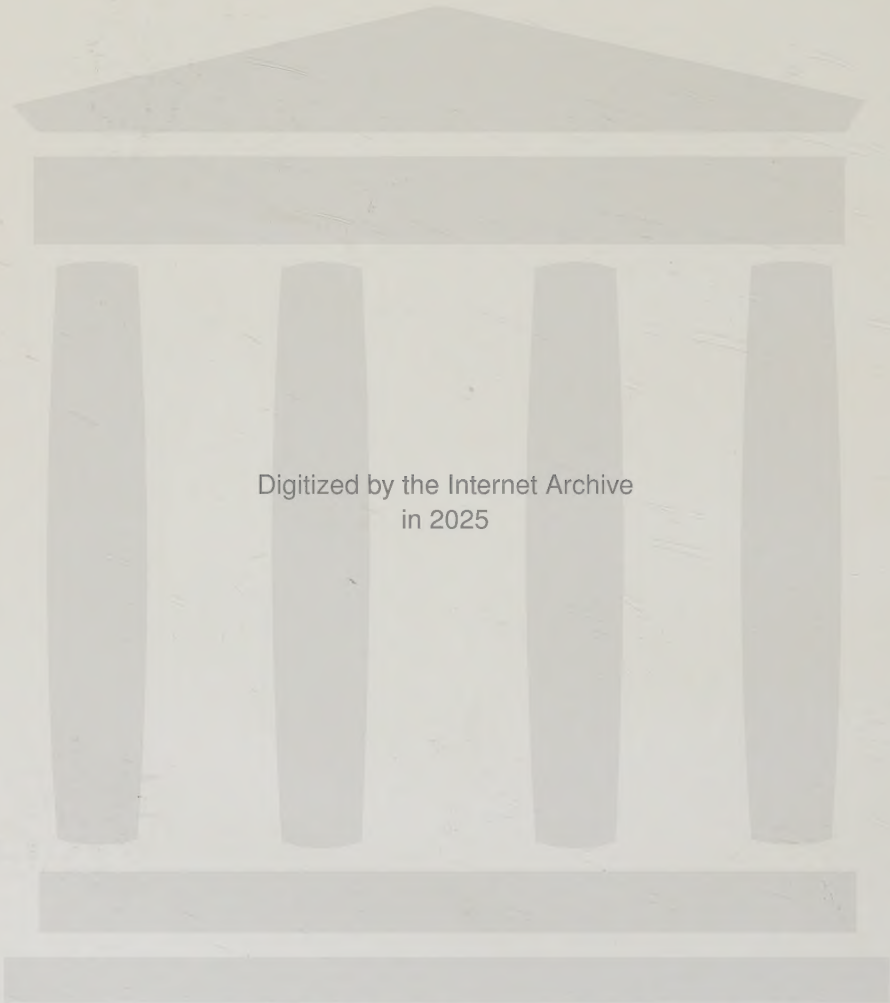
Lahore,
Dehra Dun.
Coimbatore (Tank).
Godavari (Rellagaddi).
Dacca.
Bihar (S. H. 244 and S. H. 248).
Cochin.

Morphological descriptions of the first five forms are included in Panje's " Studies in *Saccharum spontaneum* " [1933]. Except in the case of Lahore, which does not arrow at Coimbatore, both root tips and pollen mother-cells were studied. Root tips were fixed in Allen's modification of Bouin's fixative, PFA 15b [McClung, 1929] and in La Cour's 2 BD [La Cour, 1931]. Of these two fixatives, Bouin's was found more satisfactory for the study of metaphase plates. Freezing the root tips for about two to three minutes just before fixing, by surrounding them with crushed ice, had the effect of spacing the chromosomes evenly, and facilitated their counting. This is very important in a genus like *Saccharum* where the chromosomes are many.

Spikelets at the right stage were selected after preliminary examination of anthers in aceto-carmine [Belling, 1923]. They were then fixed in Allen's modification of Bouin's fixative (PFA 15b), and acetic alcohol in the proportion of one part acetic acid to three parts absolute alcohol. Materials fixed in acetic alcohol and preserved in 70 per cent alcohol proved excellent for aceto-carmine smears, even after a period of several months. An exhaust pump was used to hasten penetration.



Some Indian types of *Saccharum spontaneum*.



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SOMATIC METAPHASE IN SOME INDIAN TYPES OF *SACCHARUM SPONTANEUM* L.

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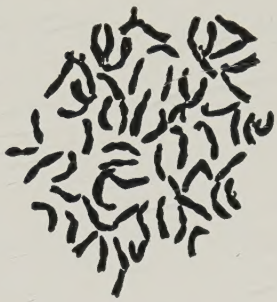

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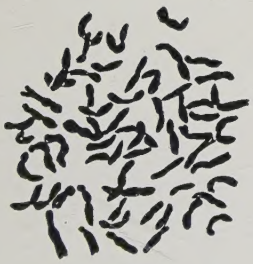

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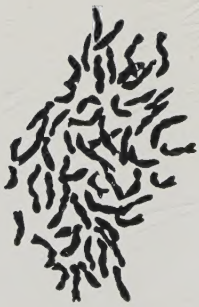

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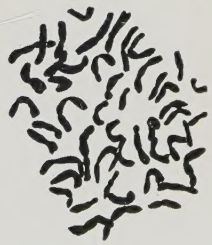

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FIG. 1. Dacca $2n=80$.

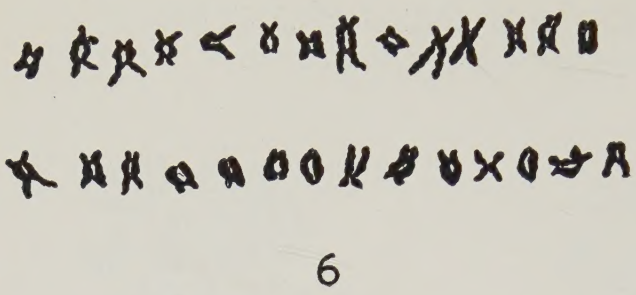
FIG. 2. Bihar $2n=64$.

FIG. 3. Rellagaddi $2n=64$.

FIG. 4. Dehra Dun $2n=56$.

FIG. 5. Lahore $2n=48$.

CHROMOSOME ASSOCIATION IN THE DEHRA DUN AND GODAVARI FORMS



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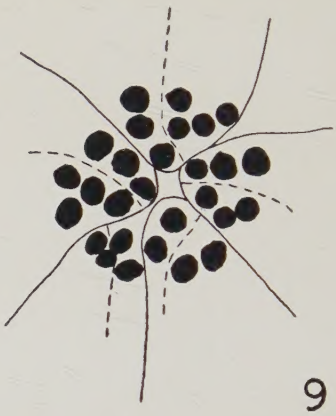

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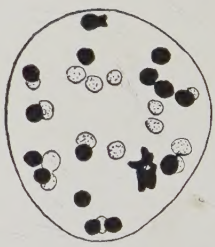

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FIG. 6. Bivalents from one P.M.C. of the Dehra Dun form $n=28$ (aceto-carmine smear).

FIG. 7. Diakinesis in the Dehra Dun form.

FIGS. 8 & 9. Prometaphase & metaphase stages in the Dehra Dun form showing "Secondary association."

FIG. 10. Early anaphase in Rellagaddi ($n=32$).

FIG. 11. Diakinesis in Rellagaddi.

FIG. 12. Prometaphase in Rellagaddi.

Sections were cut, 10μ in the case of root tips and 12 to 15μ in the case of anthers. Iron alum haematoxylin with picric acid as a de-stainer gave clearer differentiation than Iodine gentian violet.

Drawings were made at bench level with a Spencer Abbe camera lucida, a Leitz objective, 1·92 (N.A. 1·32) and a x25 eye piece, to give a magnification of 4000. For the study of aceto-carmine smears a water immersion objective (N. A. 1·2) was used.

III. The chromosome complement in *Saccharum spontaneum*

Cytological studies in *S. spontaneum* were first made by Bremer [1925] who gives the chromosome number of the Java form (Glagah) $n = 56$ and of the Celebes form " Glagah Tabongo " $n = 40$.

Chromosome counts of some of the types examined have already been published. Dutt and Rao [1932] were the first to point out that the Coimbatore form of *S. spontaneum* differs from the Java and the Celebes forms in having sixty-four chromosomes in its somatic cells. Singh [1934] reported thirty-two bivalents in the Godavari form, twenty-seven bivalents in the Dehra Dun and thirty-nine in Dacca. I find the following numbers in the types examined by me :—

	Somatic chromosomes
Lahore	48
Dehra Dun (Holes I)	56
Coimbatore (Tank)	64
Rellagaddi	64
Bihar (S. H. 244)	64
Bihar (S. H. 248)	64
Cochin	64
Dacca	80
A seedling from the Dacca form	80

The above observations suggest that the different types of *Saccharum spontaneum* represent a polyploid series with a basic number eight.

Plate II, figs. 1 to 5 give the somatic metaphase plates of the ' chromosal ' types studied. The chromosomes are found to vary both in size and morphology—at least three types can be distinguished and possibly five in some of the varieties. Both medium and secondary constrictions are found. There is indication of the occurrence of trabants in a few chromosomes of some of the varieties examined.

IV. Pollen mother-cell division

The general facts regarding meiosis are the same for all the types studied and to avoid repetition the cytology of the Dehra Dun form is given in some detail, as the one having the smallest number of chromosomes next to Lahore. This

form from Lahore—which has little or no lamina—has not so far produced any arrows in Coimbatore. It was, therefore, not possible to include in this study, observations on its meiosis.

Early prophase studies have not been attempted. Observations herein recorded have been mainly on the stages from early diakinesis or diplotene to metaphase.

(*a*) *The Dehra Dun form*

At zygotene, single threads are seen lying side by side in various grades of proximity and at diplotene loops are formed with several points of contact which are found to be ' chiasmata ' or exchange of partners between the four chromatids. An examination of early diakinesis shows the fifty-six chromosomes associated into twenty-eight bivalents by means of chiasmata (Plate III, figs. 6 and 7).

Bivalents are of two kinds, some with one chiasmata and others with two. In the case of bivalents with single chiasmata, the chiasma are localized and medium. Terminalization seldom occurs in these. They have the appearance of X's even in late diakinesis. In those with two chiasmata, terminalization is complete in the smaller chromosomes, which have the appearance of O's. Chiasmata observed at late diakinesis are never completely terminalized. Contraction of chromosomes is very intense in diakinesis. This may have been intensified by the reagent used.

With the termination of diakinetic repulsion there is a sudden convergence of the bivalents to the centre of the nucleus. From a study of large numbers of pollen mother-cells at this (pro-metaphase) stage the existence of " secondary association " of bivalents is very clear. The bivalents arrange themselves into ten groups (Plate III, fig. 8). Due partly to their close association and partly as an artefact of fixation there is generally a thin strand of linen connecting the bivalents of a group. These bivalents orient themselves to form the characteristic metaphase plate (Plate III, fig. 9). In a polar view this " secondary association " can be clearly observed. The chromosomes are very much condensed at this stage and look like spherical bodies. Inter-kinesis is short and at the second metaphase, the chromosomes are found to be still associated. Tetrad formation is normal.

(*b*) *The Godavari form* (*Rellagaddi*) $2n=64$

This is the form of *S. spontaneum* growing on the banks of the Godavari river. Its chromosome number reported by Singh [1934] is $n=32$, which I have also observed (Plate III, fig. 10). Plate III, fig. 11 shows a pollen mother-cell at late diakinesis. Thirty-two bivalents are generally observed, but in a number of nuclei examined at this stage, a single tetravalent could be observed. The number of bivalents with single chiasmata are slightly less than those in the Dehra Dun form. As pairing akes place not between chromosomes but blocks of chromosomes

CHROMOSOME ASSOCIATION IN THE BIHAR AND DACCA FORMS

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FIG. 13. P.M.C. at diakinesis stage in the Bihar form showing 28 bivalents (aceto-carmine smear).

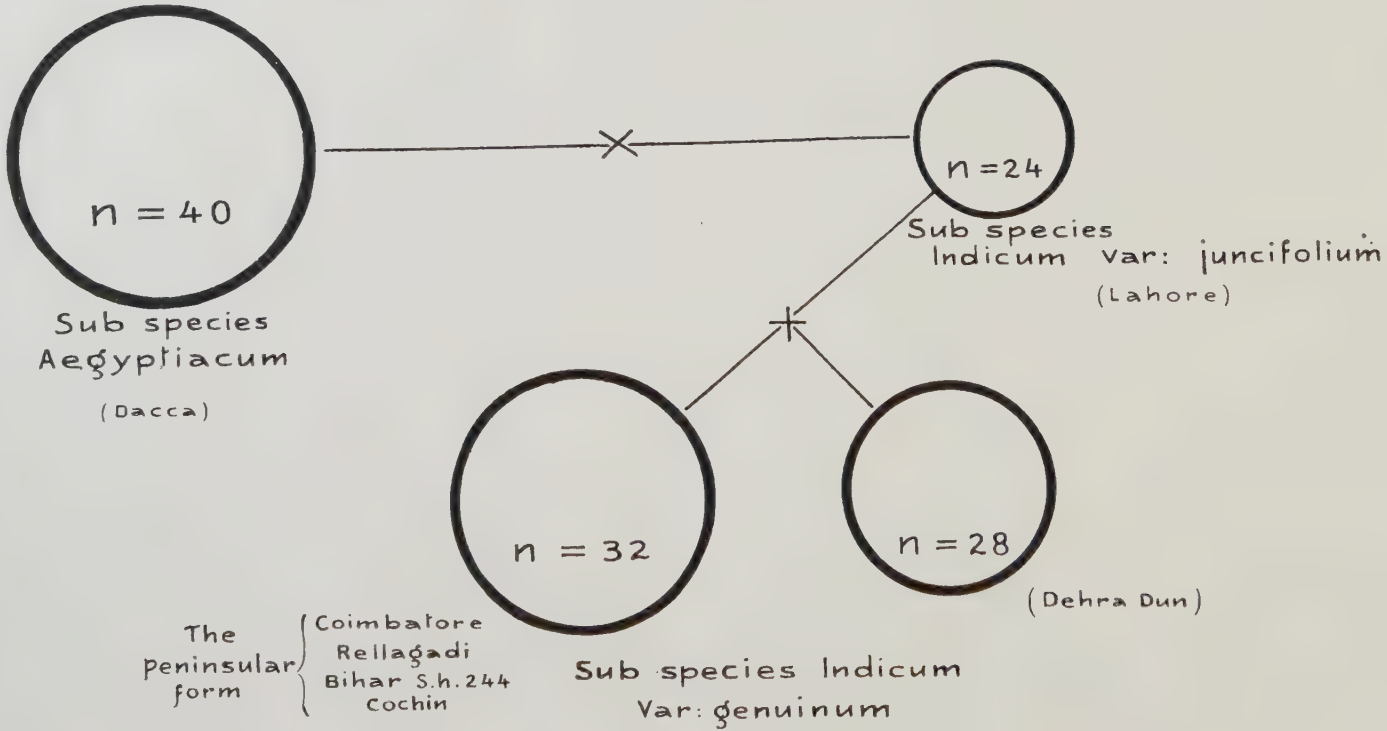
FIG. 14. Metaphase in the Bihar form showing chromosome association.

FIG. 15. Metaphase plate in the Coimbatore form.

FIG. 16. Diakinesis in the Dacca form.

FIG. 17. Metaphase in the Dacca form showing chromosome association.

DIAGRAMATIC REPRESENTATION OF THE DIFFERENT TYPES OF *SACCHARUM SPONTANEUM* IN INDIA



[Darlington, 1932], the increase in the number of chiasmata observed may be an indication of structural changes in the two chromosomes of a bivalent. The presence of a tetravalent is also noteworthy. Plate III, fig. 12 shows a pollen mother-cell at prometaphase stage. The chromosomes associate in groups of two, three and four which seems to be the prevailing type of secondary association in this variety. The later stages of meiosis are normal.

(*c*) *The Bihar form* (*S. H. 244*), $2x = 64$

This is another form in which there are thirty-two bivalents in the pollen mother-cell (Plate IV, fig. 13). No tetravalents were observed. Secondary association is more lax than in the Dehra Dun form. Ten groups were differentiated in which at least five were associations of two bivalents (Plate IV, fig. 14).

(*d*) *The Coimbatore form* (*Tank*), $2n = 64$

Thirty-two bivalents are observed at metaphase (Plate IV, fig. 15). Secondary association of bivalents is of frequent occurrence. They are associated in groups similar to the Bihar form (S. H. 244) and about ten groups could be traced. A number of bivalents with single chiasma, which remain unterminalized, was observed at diakinesis giving a configuration like X.

(*e*) *The Dacca form* $2n = 80$

Plate IV, fig. 16 shows a reconstructed nuclei from two sections of a pollen mother-cell at diakinesis. Variation in length of chromosomes is very marked in this variety. In particular a number of very small chromosomes are found to associate in groups at pro-metaphase. "Secondary association" is very marked (Plate IV, fig. 17) and the groups are composed of two, four and five bivalents. An analysis of the groups shows that in a number of pollen mother-cells the forty bivalents arrange themselves into four groups of five, four groups of four and two groups of two, making in all ten groups.

V. Evidence of hybridity within the species

Secondary polyploidy.—It would appear from the chromosome counts of the various clones examined that the species *S. spontaneum* represents a polyploid series. The association of more than two chromosomes at metaphase of meiosis was first observed by Kuwada [1910] in tetraploid *Oryza sativa*. This phenomenon has since been observed in many polyploids. Except for the single tetravalent observed in Rellagaddi, no multivalents have been observed so far in the Indian species. Its absence goes to prove that polyploidy in this species must have occurred through hybridization and not through somatic duplication of chromosome sets, as in auto-polyploids like *Tulipa*.

Besides the formation of multivalents, which is the result of prophase pairing, a secondary or post-synaptic association of bivalents, arising from a general affinity of relatively homologous bivalents, was first observed by Darlington [1926] in Prunus. It is generally observed in plants with small chromosomes. Lawrence [1932] has shown that "secondary association" is of wide occurrence in many species and provides "valuable criterion for homology in hybrid polyploids". We have in *S. spontaneum* strong evidence of this post-synaptic association. The bivalents generally associate into groups of five or ten, which is the basic number of other Andropogonae like *Sorghum*, *Erianthus*, *Imperata* and *S. officinarum*.

In *S. spontaneum* (Dehra Dun) the eighteen bivalents arrange themselves into ten groups as follows :—

Five groups of two bivalents
Two groups of three bivalents
Three groups of four bivalents

There is also an indication of a closer affinity between two of these ten groups so that in some nuclei a more compact association into five groups may be observed. If *A*, *B*, *C*, *D* and *E* represent the basic chromosomes, we can consider the gametes in the Dehra Dun form as being made up approximately of the following chromosome complement :—

1. *A.A.A* + *A.A.A.A.*
2. *B.B.B* + *B.B.B.B.*
3. *C.C* + *C.C.C.C.*
4. *D.D* + *D.D.*
5. *E.E* + *E.E.*

We thus see that the twenty-eight bivalents are composed of two groups of sixteen and twelve bivalents respectively. Since these numbers correspond to the haploid number of the Peninsular forms with thirty-two chromosomes and the Lahore form with forty-eight chromosomes, the Dehra Dun form may be a hybrid between these two chromosome types.

The presence of five pairs of bivalents in the Peninsular form, which stand apart from the rest, seems to indicate that this variety is also a product of hybridization. It is highly probable that the thirty-two chromosome forms have arisen as natural crosses between a type like Dacca ($n = 40$) and a type like Lahore ($n = 24$). In its morphological characters the Peninsular form is found to be intermediate between the wire-leafed "Lahore" and the broad-leafed "Dacca". The range of variation found in this group is also very wide which may be an additional evidence for its being considered a hybrid.

Chiasma behaviour.—The chromosomes of *S. spontaneum* examined fall into two types from the point of chiasma behaviour.

Type I with a single chiasma which is localized.

Type II with one or two chiasmata which are formed at random.

The latter have a fairly high frequency of terminalization.

The presence of bivalents with greater frequency of chiasmata in proportion to their length is noteworthy (Plate III, fig. 6). This is in accordance with the "chiasma theory of pairing" of Darlington. If the frequency of pairing is proportional to length, some of the shorter bivalents would be unpaired at metaphase which would then lead to irregularity in germ formation and thus interfere with sexual reproduction of the species. This property may thus be considered as a genetic factor influencing the behaviour of chromosomes [Darlington, 1932].

This differential behaviour in the chiasma formation of the *S. spontaneum* may be taken as another evidence of its hybridity. This will be dealt with more fully in a separate paper.

VI. Conclusions

Barber, in his "Studies of *Saccharum*" [1918], found the broad-leafed form from Dacca to resemble cultivated canes more than any of the other Indian forms examined by him. This form is now found to have the same number of chromosomes as *Saccharum officinarum*. The discovery of other types with $n = 40$ chromosomes, like *S. robustum* from New Guinea and Glagah Tabongo, from Celebes points to this form having a wide distribution in the tropics. From a study of its morphological characters, the Dacca form comes nearest to Hackel's subspecies, *ægyptiacum*.

It seems that the wire-leafed form from Lahore with $2n = 48$ represents a form of *Saccharum spontaneum* having a basic number six or twelve instead of the usual five or ten found in other Andropogonae. There is strong indication that the forms with thirty-two and twenty-eight chromosomes, which represent the "intermediate" forms of Hackel, are natural hybrids or their back-crosses, between the two types ($n = 40$) and ($n = 24$). These intermediate forms, because of their wide distribution, rightly represent the sub-species '*indicum*', variety "*genuinum*" of Hackel. They show great variation in vegetative characters and are best suited for the study of the differentiation of the species from an ecological point of view. The wire-leafed form, which Hackel describes as subspecies *indicum*, variety *juncifolium*, deserves to be classed as a separate species perhaps "*Saccharum juncifolium*", because of its distinctive morphological characters and its unique chromosome complement.

It is highly probable that the more primitive types of *S. spontaneum* are those with lesser number of chromosomes. These studies on Indian forms

indicate that the primitive forms occur in India. It is likely that a more comprehensive study of the species will lead to the discovery of more "ecotypes" with chromosome number forty-eight and fifty-six than those hitherto examined.

VII. Summary

The Indian types of *S. spontaneum* form a polyploid series with the following chromosome numbers :—

$2n = 48$
$2n = 56$
$2n = 64$
$2n = 80$

2. The somatic chromosomes show primary and secondary constrictions. They also vary in length.

3. Pairing of chromosomes at meiosis is by means of chiasmata. Chromosomes are always associated as bivalents except in Rellagaddi in which a single tetravalent was observed.

4. The number of chiasmata observed is not always proportional to length of chromosomes.

5. "Secondary association" of bivalents is observed. The chromosomes arrange themselves into five or ten groups which is the basic number of the Andropogonae.

6. There is evidence of hybridity within the species. Of the Indian forms examined, the type with forty-eight chromosomes represents Hackel's variety "*juncifolium*" and the type with eighty the variety "*ægyptiacum*". Those with intermediate numbers seem to be natural hybrids between these two forms.

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Burma variety of *Saccharum spontaneum*.

CHROMOSOME NUMBERS IN A BURMA *SACCHARUM SPONTANEUM* L. & ITS SEEDLINGS

FIG. 1. Somatic metaphase from the root tip of the parent Burma *S. spontaneum* $2n=96$.

FIGS. 2–6. Somatic metaphases from the root tip of some seedlings of the Burma form. $2n=96$; $2n=108$–110; $2n=112$–114; $2n=114$; $2n=124$; respectively.

CYTO-GENETIC ANALYSIS OF *SACCHARUM SPONTANEUM* L.

2.—A TYPE FROM BURMA

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(With Plates VI-VIII)

According to Barber [1918] *Saccharum spontaneum* in Burma represents a series of forms ranging from a thick "Land form" to an aquatic type like the Dacca form of India. The only Burma variety of *S. spontaneum* in the collection at the Imperial Sugarcane Station is from a clone from Mandalay collected in 1929. This variety is the most outstanding of the *S. spontaneums* grown at Coimbatore. It attains a height of fifteen to eighteen feet, propagates very profusely by means of underground stems and has a splendid erect habit (Plate VI). The stem has a much longer internode than any of the other *S. spontaneums*. Panje [1932] notes a number of other morphological characters in which this form differs from the Indian and the East Indies types studied by him. The plant flowers very rarely in Coimbatore and only a single inflorescence was observed in 1933.

Cytological observation.—As the plant did not arrow in 1934-35, only root tips were available. These were fixed in Allen's modification of Bouin's PFA-15b after freezing, sections cut at 10μ and stained with Iron Alum haematoxylin with picric acid as de-stainer. The metaphase plate of somatic division showed ninety-six chromosomes (Plate VII, fig. 1). This is the first record of this number for any type of *S. spontaneum* studied. The chromosomes appeared similar to those of the Indian *S. spontaneum* studied, in having both primary and secondary constrictions. The prevalence in India of forms of *S. spontaneum* with chromosome $2n=64$ [Dutt and Rao, 1933 and Singh, 1934] and the occurrence in Sumatra of a "gigas" form with $2n=124$ [Singh, 1934] points to the Burma form being a triploid with basic chromosome number $x=32$.

Triploids occur in nature either as the result of a cross between diploids and tetraploids of the same species or as the result of the failure of reduction in the archesporium of one of two diploid parents and the consequent fusion of an x with an unreduced $2x$ germ cell. According to Bremer [1929] doubling of chromosomes in the female parent is a common phenomenon in *Saccharum*. The regionally intermediate Burma form might be either a natural cross between an Indian form with $n=32$ and a tetraploid form like the "gigas" form of Sumatra (Plate VIII) or it may represent the product of fertilization of an abnormal diploid gamete with a haploid one as noted by Bremer in many other *Saccharum* species. Cytological evidence from pollen mother-cell studies is necessary to throw further light on the origin of this interesting Burma form. Its possible position amongst other *S. spontaneum* is shown in the diagram.

Progeney of the Burma form.—The single arrow produced in 1933—and left unbagged—yielded a number of seedlings which showed marked differences in morphological characters. Many of these were thicker than the parent and looked like very medium sugarcanes. Root tips of some of these were examined and they showed 96 to 124 chromosomes at somatic metaphase (Plate VII, figs. 2-6). As the arrow was unbagged, and consequent possibility of cross-pollination, much value cannot be placed on the variation observed in their chromosome numbers. However the numbers found fall within the expected frequency of chromosome distribution in a triploid with $2n=96$.

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DIAGRAMATIC REPRESENTATION OF THE DIFFERENT TYPES OF *SACCHARUM SPONTANEUM*

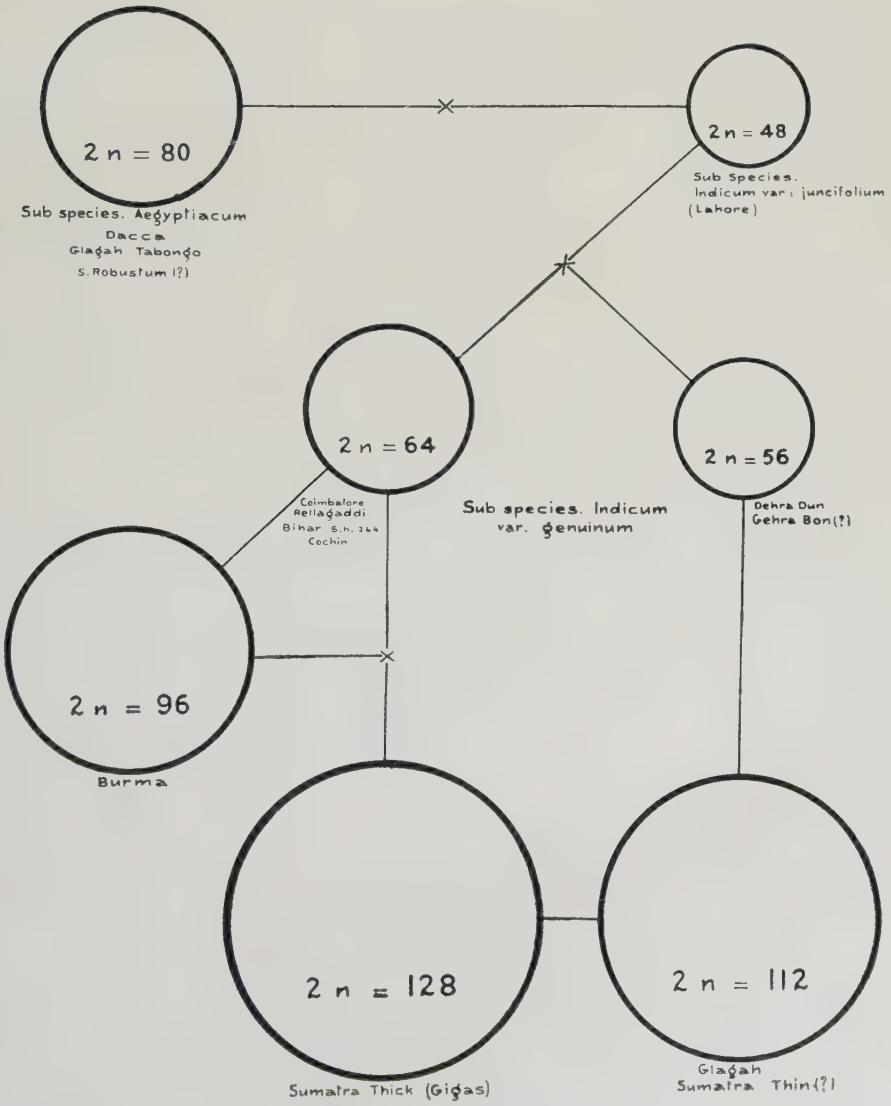

Fig. 1. Healthy seedling.
Figs. 2-5. Seedlings showing various stages of the disease.
Fig. 6. Cane leaf showing diffused patches.
Fig. 7. Wheat leaf showing definite spot formation.
Fig. 8. Barley leaf showing closely arranged stripes.

SOME NEW SEEDLING DISEASES OF SUGARCANE

BY

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(With Plates IX-XI)

During March 1934 sugarcane seedlings growing in pans at the Cane Breeding Station at Coimbatore were found to die in large numbers. The disease was especially widespread in the thick cane seedlings. The first indication of the disease in the pans was the appearance of a number of seedlings wilting or dying in small patches. Sometimes only one or two pans in the same culture showed the disease whilst others remained free. In the dying seedlings root development was very poor, and in some cases the roots were very soft, rotten and discoloured. In a few cases the roots had developed well, but the tissues round the collar showed brownish discoloration and the leaf tips were drying downwards. Sometimes the collar had simply become soft without any brownish discoloration. A species of *Helminthosporium* was found growing profusely in the dead seedlings.

In January 1935 all the new sowings were again examined and the following fungi were found in the dying seedlings. Two species of *Helminthosporium*, *Pythium* sp., *Fusarium* sp., *Alternaria* sp., *Nigrospora* sp., and a sclerotial fungus. *Helminthosporium* spp. and *Pythium* sp. were most prominent. The present paper deals with two species of *Helminthosporium* and a *Pythium* sp. which were found to be parasitic.

I. *Helminthosporium* SPP.

Symptoms.—The disease usually appears in the early stages of the growth of the seedlings, forming small, narrow, reddish stripes, crowded together but not forming definite spots with a margin. When older they are bright to dark brown in colour. The leaf-sheaths become dark brown or olivaceous owing to the formation of numerous conidiophores and conidia.

Morphology.—The mycelium consists of septate, subhyaline hyphae, both inter and intra-cellular, which ramify in the tissues. Haustoria have not been found. The hyphae are 2 to 4μ in breadth. Conidiophores are formed in the discoloured portion of the leaf-sheath and emerge through the epidermal cells

singly or in pairs. They are unbranched, brown to dark olivaceous, 2 to 5 septate, 40 to 130μ in length and 5 to 7μ in breadth. The basal cell of the conidiophore is slightly swollen. The geniculation associated with the production of successive conidia is well defined. Conidia are borne singly at the tip of the conidiophores, and as successive ones are being formed the tips become curved owing to the large number of geniculations, size 20-140·5μ × 9-16·5μ, average 62 × 13·5μ 3 to 11 septate not constricted at the septum. The end septa are deeply marked; spores are dark brown, end cells lighter in colour, tapering towards both ends with a prominent hilum. They germinate by producing germ tubes from either end cell and sometimes from the intermediate cells also.

The following inoculation experiments were carried out to test pathogenicity.

TABLE I

Results of experiments carried out to test the pathogenicity

Date	Host	Place of inoculation	Result	Control
24th May 1934 .	Co. 213 cane .	4 shoots inoculated in different places.	+	Free
29th May 1934 .	Co. 213 cane .	8 shoots, leaves . .	+	Do.
28th June 1934 .	Co. 213, 416, 419, 420, M16, B3412 Lalgirah cane.	Plants in pots. Leaves inoculated in different places. Plants moist by spraying with water.	+	Do.
25th Feb. 1935 .	Wheat . .	30 seedlings ; leaves . .	+	Do.
	Barley . .	30 seedlings ; leaves . .	+	Do.
14th May 1935 .	Maize . .	6 plants ; leaves . .	+	Do.
	Jowar . .	6 plants ; leaves . .	+	Do.
	Co. 213 cane .	6 shoots ; leaves . .	+	Do.
31st May 1935 .	Co. 213 cane .	6 canes, leaf and leaf-sheaths in different places.	+	Do.

This species gives a very high percentage of infection not only with different varieties of cane but also with maize, *jowar*, wheat and barley. On wheat it forms definite spots with a grey centre and reddish brown margin similar to those of *Helminthosporium sativum* P. K. & B.

FIG. 1. Diseased and healthy seedlings.

FIG. 2. Conidia of *Helminthosporium halodes* (×250).

The disease has so far been observed only in the seedlings. When selected seedlings from diseased pans are transplanted in the field, they do not show any symptoms of the disease.

Identity of the fungus.—The measurements of this fungus together with the degree of septation and shape of spores agree very closely with that of *H. halodes* described by Drechsler [1923] on the leaves of *Distichlis spicata* (L.) Greene, from New York. Mitra [1931] described *H. halodes* var. *tritici* on wheat from Central Provinces. He succeeded in infecting leaves of maize, *jowar*, wheat, barley, oats, rice, and cane with this strain. The measurements of these three fungi are given below for comparison :—

TABLE II

Measurements of the fungi

Fungus	Spore length	Spore width	Septa
H. halodes	20-103μ	10-14μ	1-12
H. halodes var. *tritici*	23-73μ	13-20μ	2-9
Isolation from cane seedlings	20-104·5μ	9-16·5μ	3-11

It will be seen that the cane strain of *Helminthosporium* agrees in size and septation of the spores more closely with *H. halodes* than with *H. halodes* var. *tritici*.

This is the first record of its occurrence on sugarcane.

In addition to *H. halodes* another species of *Helminthosporium* has been isolated from sugarcane seedlings which agrees with the description of *Helminthosporium tetramera* described by MacKinney [1925] from Oklahoma, U. S. A., where it is found associated with foot-rots of wheat. Infection experiments carried out proved that this fungus is a weak parasite when compared to *H. halodes*.

II. *Pythium* SP.

The roots of cane seedlings become soft and rotted, the collar flaccid and discoloured and the seedlings collapse near the ground level. When seedlings are pulled out the rotten roots are left behind in the soil.

Infection experiments carried out to prove its pathogenicity are summarised below :—

TABLE III

Results of infection experiments

Date	Plant	Where inoculated	Infection	Control	Remarks
6th July 1935	Sugarcane .	5 seedlings in water culture ; inoculated at roots.	+	Free	
	Maize . .	Do. .	+	Do.	Slight infection.
	Jowar . .	Do. .	+	Do.	Do.
27th July 1935	*Carica papaya* .	Fruits . .	—	Do.	Fungus entered through wound in *Carica papaya* and *Lagenaria vulgaris*, but infection did not spread.
	Hibiscus esculentus.	Do. . .	—	Do.	
	Trichosanthes dioica.	Do. . .	—	Do.	
	Lagenaria vulgaris.	Do. . .	—	Do.	
	Amaranthus sp.	Stem . . .	—	Do.	
3rd Aug. 1935	Cane, maize, *jowar.*	6 seedlings each, in water culture ; kept at 25°C.	+	Do.	
5th Aug. 1935	Wheat and barley.	40 seedlings each, in sand culture ; kept at 25°C. infected at collar.	+	Do.	Very active infection, collar becomes soft, brown and collapses.
6th Aug. 1935	Maize . .	6 seedlings growing in sand ; infected at the collar.	+	Do.	Slight infection.
	Jowar . .	Do. . .	+	Do.	Do.
13th Aug. 1935	Sugarcane .	3 shoots in pots ; culture placed touching the leaf-sheath.	+	Do.	
	Maize . .	Roots floating in water ; culture placed touching the roots.	+	Do.	Very slight infection.
16th Aug. 1935	Wheat . .	40 seedlings in sand ; culture placed at collar ; kept at 25°C.	+	Do.	Very active infection.
2nd Sept. 1935	Wheat, barley and oats.	20 seedlings each.	+	Do.	Do.

FIG. 1. Conidia of *Helminthosporium tetramera* (×250).

FIG. 2. Conidia and oospores of *Pythium graminicolum* (×250).

When cross-inoculated, the fungus attacks maize, *jowar*, wheat, oats and barley in addition to sugarcane. On maize and *jowar* it is not very active but on wheat, oats and barley seedlings it is very vigorous and kills the seedlings in three days. The collar and the roots become soft and discoloured brown or yellowish brown. The mycelium is found in all the tissues. Later on oospores develop in large numbers in the epidermal tissues.

The measurements of sporangia, zoospores and oospores of the strain from cane seedlings agree in all respects with those of *Pythium graminicolum* described by Subramaniam [1928] on wheat from Dharwar, Bombay. Matthews [1931] isolated *Pythium graminicolum* from a sandy soil in North Carolina in which Sarracenia was growing.

The measurements of the three strains are given below for comparison :—

TABLE IV

Measurements of the strains

Fungus	Host	Oogonia	Average	Oospores,	Average
Pythium graminicolum.	Wheat .	16·5-28·6μ	..	15·4-16·4μ	
Do. .	Sarracenia	24-36μ	30μ	21·6-32μ	28μ
Do. .	Sugarcane	16·9-33μ	25μ	16·3-10·8μ	23μ

This is the first record of *Pythium graminicolum* on cane seedlings in India.

III. CONTROL MEASURES

The seeds of the susceptible variety P. O. J. 2725 × Co. 243 were obtained from Coimbatore and sown at Pusa, where the disease also developed. It is therefore presumed that the foot-rot disease is seed-borne. Treatment of the seeds and soil with a solution of 0·25 per cent Uspulun checked the disease to a large extent both at Coimbatore and Pusa, very few seedlings dying in the treated pans. When the diseased seedlings were removed and the rest were allowed to grow, they remained healthy throughout the period they were kept under observation.

So far the disease has been observed only on very young seedlings. Should it become a serious source of inconvenience, it can be controlled by application of suitable fungicides to seed and soil.

Summary

Helminthosporium halodes, and *H. tetramera* have been found to be the cause of seedling diseases of sugarcane. *H. halodes* is an active parasite, while *H. tetramera* is a weak one. These two fungi cause foot-rot of the seedlings, and are here recorded for the first time on sugarcane.

Pythium graminicolum has been found on sugarcane for the first time in India as the cause of a seedling disease. This fungus when cross-inoculated attacks cane, wheat, oats and barley very vigorously and maize and *jowar* to a slight extent.

Acknowledgments

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A BIOCHEMICAL STUDY ON THE DECOMPOSITION OF FARMYARD MANURE AND SULPHATE OF AMMONIA IN THE PADDY SOILS OF THE CENTRAL PROVINCES*

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INTRODUCTION

The decomposition of sulphate of ammonia or farmyard manure in different soils under normal arable conditions has been widely studied ; but comparatively little is known regarding the chemical and microbiological processes involved in the decomposition of such fertilisers under the usual water-logged conditions of the paddy field.

There are some scattered references in the literature to nitrogen changes in submerged soils due to the addition of nitrogenous fertilisers. Warington [1892] for the first time detected in some laboratory experiments a loss in added nitrates when the soils were kept water-logged. Daikuhara and Imasaki [1907] also made similar observations. Nagaoka [1904] was the first to report the formation of nitrite when nitrates were added to paddy fields and Kelley [1911] stated that nitrites so formed were toxic to paddy seedlings. In a recent paper Kapp [1933], however, observed that in Arkansas soils application of sodium nitrate and ammonium sulphate does not lead to accumulation of nitrite in submerged rice soils in sufficient quantities to prove toxic to the seedlings. According to Oelsner [1918] nitrates as well as total nitrogen are lost on water-logging the soil. Subrahmanyan [1927] failed to observe any such diminution in total nitrogen although he noticed that in submerged soils there was a slight diminution in nitric nitrogen and a distinct increase in free and saline ammonia. According to Harrison and Aiyer [1913-14] nitrification is impossible in sub-merged paddy soil and the nitrates produced during the dry season are quickly denitrified so that the nitrogen required by the crop is obtained from the ammonia and nitrogenous organic compounds produced by the anaerobic decomposition of the proteins of added green manure.

* The investigation recorded in this paper was carried out at the Bio-Chemical Laboratory of the Central Provinces Rice Research Scheme financed by the Imperial Council of Agricultural Research.

The work described in this paper was mainly undertaken with the objects of ascertaining firstly, the relative effect of the decomposition of farmyard manure and sulphate of ammonia under water-logged conditions on the nitrogen contents and microflora of two of the typical rice soils of the Central Provinces and secondly, to what extent the results vary fundamentally from those obtained under aerobic soil conditions.

Experimental

The soils used for these experiments were "*Matasi*" (sandy loam) and "*Dorsa*" (medium clay) from unmanured rice plots on the Government Experimental Farm at Labhandi, Raipur. Geologically, these soils are formed entirely upon Pre-Cambrian rocks of the Purana and Archaean groups. Their chemical and mechanical compositions are given below.

Table I

Chemical and mechanical compositions of Matasi and Dorsa soils

(*a*) *Chemical analysis*

	Matasi	*Dorsa*
	Per cent	Per cent
Insoluble silicate and sand	87·41	77·78
Alumina	4·74	11·43
Lime	0·62	0·39
Potash	0·46	0·86
Phosphoric Acid	0·02	0·03
Magnesia	0·30	0·81
Loss on ignition	6·70	7·80
Carbon	0·56	0·56
Total	100·81	99·66
Total nitrogen	0·05	0·06
Available phosphoric acid	0·001	0·001
Available potash	0·10	0·11
pH value	6·8	7·2
C/N	11·2	9·3

(*b*) *Mechanical analysis* (*By Puri's method*)

Soil	Clay	Fine silt	Coarse silt	Sand	Total
	Per cent	Per cent	Per cent	Per cent	
Matasi	25·36	3·70	8·84	58·74	96·64
Dorsa	48·26	5·08	7·86	34·86	96·06

A series of comparative tests on the changes in bacterial numbers, ammonia, nitrate and nitrite contents, carbon dioxide evolution and nitrate-reducing capacity in culture solutions were carried out in each of these soils receiving farmyard manure (well-rotted cattle-dung), sulphate of ammonia or no nitrogen under water-logged and aerobic conditions.

The principle of the technique employed is the same as communicated in a preceding paper by one of the writers [Mukerji, 1932] for following the biochemical changes in soils under conditions approximating more closely to those of the field than are obtained in the conventional tumbler technique or in pot cultures. Air-dried samples of soils (0—6 in.) were passed through one-mm. sieve, and 1000-grm. lots uniformly moistened to fifty per cent of the water-holding capacity of the soil by applying as a spray about 200 c.c. of water. In a parallel series the soils were maintained under water-logged conditions by having a film of water always standing over the surface. Wide-mouthed glass jars (two-litre capacity) with one kg. of soil were set up under the two different moisture conditions with soil receiving farmyard manure, sulphate of ammonia or no nitrogen. The manures were applied at the rate of 100 lb. nitrogen per acre and their doses per kg. of soil calculated on the basis of one acre of soil weighing approximately two million lbs.

For each treatment two jars were set apart. The lower ends of the jars were cut off from light by dark paper shades. Aerobic and submerged cultures were sampled for analysis on alternate days. The sampling which was done by boring with a small augur involved thorough mixing of the soils and constant renewal of the air over the soil mass. The jars were shaken every day for a few minutes to provide free exchange of gas between the soil particles, supernatant water (in water-logged cultures) and the air above the soil.

Soil samples were withdrawn from each individual jar every week, and bacterial numbers, ammonia, nitrate and nitrite determined. Matthews' aeration method was used for the determination of ammonia. Nitrate and nitrite were estimated by Harper's phenol-disulphonic acid method and Griess-Ilosva method, respectively. Bacterial counts were made by plating on Thornton's agar medium. Besides ammonifying organisms this medium is also capable of growing rapidly, as has been reported in a previous publication by one of the authors [Mukerji, 1931], some species of nitrite forming bacteria which differ widely from *Nitrosomonas* or *Nitrosococcus*.

BACTERIAL NUMBERS AND NITROGEN CHANGES

The bacterial counts are given below.

TABLE II

Average bacterial numbers in millions per grm. of soil

(*The means of the replicate counts for each treatment have been assigned with their respective probable errors.*)

Treatments	*Dorsa* soil — Days from commencement							*Matasi* soil — Days from commencement					
	0	7	14	28	42	49	56	0	7	14	21	28	35
Farmyard manure—													
AE	15·0 ‡2·7	10·1 ‡1·8	24·2 ‡2·0	9·4 ‡1·9	9·4 ‡1·8	19·6 ‡2·1	18·4 ‡2·2	22·9 ‡1·3	26·8 ‡1·8	25·1 ‡2·0	21·9 ‡0·9	14·3 ‡2·1	10·7 ‡1·0
WL	15·0 ‡2·9	11·9 ‡0·9	16·8 ‡1·9	5·4 ‡1·0	1·2 ‡0·4	6·0 ‡0·5	6·1 ‡1·9	22·9 ‡1·5	18·6 ‡2·1	15·3 ‡1·4	10·1 ‡2·3	4·0 ‡0·6	7·4 ‡2·1
Ammonium sulphate													
AE	13·8 ‡0·9	19·1 ‡2·2	15·1 ‡0·7	5·9 ‡1·3	22·3 ‡1·6	33·6 ‡2·1	27·7 ‡1·6	3·8 ‡0·5	29·5 ‡2·1	19·3 ‡1·3	15·8 ‡1·1	11·1 ‡0·9	14·3 ‡1·2
WL	13·8 ‡1·6	16·2 ‡2·1	8·6 ‡0·7	6·5 ‡0·7	3·0 ‡0·4	7·9 ‡2·6	5·8 ‡1·9	3·8 ‡0·7	20·0 ‡1·6	17·7 ‡1·0	14·7 ‡1·0	6·8 ‡0·4	11·8 ‡1·5
No nitrogen—													
AE	16·3 ‡2·1	38·3 ‡1·2	31·5 ‡1·0	13·6 ‡2·4	18·9 ‡2·2	28·9 ‡0·8	33·1 ‡1·9	9·8 ‡1·2	44·9 ‡3·1	16·7 ‡0·8	17·6 ‡1·7	7·8 ‡0·9	8·9 ‡0·8
WL	16·3 ‡1·0	14·7 ‡1·7	15·9 ‡2·3	5·3 ‡1·9	11·4 ‡2·1	9·5 ‡1·4	6·8 ‡0·8	9·8 ‡1·4	34·5 ‡3·0	16·7 ‡1·0	10·1 ‡0·7	5·1 ‡0·7	4·1 ‡0·8

AE = Aerobic.
WL = Water-logged.

It will be observed that in *Matasi* soil, both under aerobic and water-logged conditions, the general level of the bacterial population with sulphate of ammonia and no nitrogen was low at the commencement but rose remarkably during the first week ; the numbers subsequently decreased with minor fluctuations. With farmyard manure the population was maintained at a uniform high level from the very beginning to the end of the third week, afterwards there was a gradual decrease. No such uniform change was noticed in *Dorsa* soil in which the bacterial numbers, although fairly high at the outset, showed only periodic rise and fall. The bacterial numbers in the farmyard manure and sulphate of ammonia treated cultures of this soil were distinctly lower than in the control. This was confirmed, as shown later, by the respective amounts of carbon dioxide evolved from *Dorsa* soil receiving all these treatments. The depressing effect of farmyard manure on the bacterial population in the heavier of the two soils is somewhat surprising, and is apparently due to the formation of some intermediate product which proves toxic to the organisms capable of growing on Thornton's medium.

The means of five or more counts at seven-day intervals for the whole experiment are given below.

TABLE III

Means of bacterial numbers at seven-day periods

Treatments		*Dorsa* soil (No. in millions per grm. of soil)	*Matasi* soil (No. in millions per grm. of soil)
Farmyard manure	Aerobic	15·2	20·3
	Water-logged . . .	8·9	14·1
Ammonium sulphate	Aerobic	19·6	15·6
	Water-logged . . .	8·8	12·5
No nitrogen	Aerobic	26·2	16·1
	Water-logged . . .	13·4	11·4

It is obvious that the relative effect of the individual manurial treatment on the bacterial population is not the same in the two soils.

On comparing the aerobic cultures among themselves it is found that farmyard manure gave in general the highest bacterial counts in *Matasi* soil and the lowest in the heavier soil—*Dorsa*. Sulphate of ammonia also depressed the population very markedly in *Dorsa* soil, whereas in *Matasi* soil it did not bring about any appreciable change in the bacterial numbers.

Under water-logged condition *Matasi* soil did not show any marked difference in bacterial counts between the farmyard manure, sulphate of ammonia and no nitrogen treatments. But in *Dorsa* soil under similar condition the numbers in the farmyard manure or sulphate of ammonia treatment were distinctly less than those in the control.

The water-logged cultures in both the soils gave consistently lower numbers than the aerobic ones. This is according to expectation, since the organisms counted in the present case were mostly aerobic. One important fact that emerges out of the plate counts is that strictly aerobic bacteria are present in surprisingly large numbers under anaerobic condition in water-logged soils—thus doubtlessly proving the presence of a fairly abundant supply of oxygen in the rice soils during the crop season.

Table IV gives for each treatment the mean differences in bacterial numbers between the aerobic and water-logged cultures.

TABLE IV

Mean differences in bacterial numbers between aerobic and submerged cultures

Treatments	*Dorsa* soil (millions per grm. soil)	*Matasi* soil (millions per grm. soil)
Farmyard manure	6·3	10·4
Ammonium sulphate	10·8	3·5
No nitrogen	14·9	4·6

It is interesting to note that both sulphate of ammonia and no nitrogen water-logged treatments depressed the bacterial population as determined by the plate counts more in *Dorsa* soil than in *Matasi*. Farmyard manure, on the contrary, caused the least difference in bacterial numbers between the aerobic and water-logged cultures in *Dorsa* soil and the highest in *Matasi*.

The results of ammonia determination in both the series are detailed in Table V.

TABLE V

Ammoniacal nitrogen changes (Ammonia-N as parts per million)

Treatments		*Dorsa* soil									*Matasi* soil					
		Days from commencement									Days from commencement					
		0	7	14	21	28	35	42	49	56	0	7	14	21	28	35
Ammonium sulphate	AE	80·6	79·4	46·8	61·9	86·6	102·1	65·9	39·8	49·3	141·5	80·0	119·0	87·2	91·1	82·4
	WL	80·6	58·3	74·4	74·5	64·8	59·0	75·7	45·7	52·6	141·5	69·0	72·5	55·1	84·3	112·1
Farmyard manure	AE	48·2	32·3	96·1	22·8	45·9	125·2	48·4	47·7	32·8	105·5	72·7	29·4	85·4	66·0	90·2
	WL	48·2	20·7	51·5	57·8	51·2	78·7	53·2	35·5	35·4	105·5	51·4	57·0	46·1	56·3	108·6
No nitrogen	AE	33·6	30·1	31·1	53·3	57·9	51·0	46·0	43·7	48·6	90·9	83·0	99·1	97·2	88·4	141·0
	WL	33·6	19·9	31·1	51·1	59·1	54·1	62·6	42·1	44·8	90·9	68·7	83·0	141·2	48·7	104·1

AE = Aerobic.
WL = Water-logged

Proper precautions were taken in using Mathews' method for these determinations. A water pump was used for aeration ; acid sprays were guarded against and much care was taken to ensure that the optimum amount of air passed through the aerating tubes.

It may be observed from the data given above that in *Matasi* soil water-logging led to a diminution in ammonia both in the sulphate of ammonia and no nitrogen treatments. A similar effect, although not so marked, is also noticeable in the case of farmyard manure treatment. In *Dorsa* soil while there was no appreciable change in ammonia as a result of water-logging in culture receiving either farmyard manure or no nitrogen, a slight increase in ammonia took place in the one to which sulphate of ammonia was applied.

The rate of decomposition of farmyard manure under submerged condition approximates to that under aerobic condition—more in *Dorsa* than in *Matasi* soil. The results of the bacterial counts also lead to the same conclusion.

The figures for the accumulation of ammonia in the sulphate of ammonia treatments indicate that in *Matasi* soil the reduction of ammonia under submerged condition is more rapid than its oxidation in the aerobic culture. The reverse is, however, the case in the clayey soil—*Dorsa* in which the effect of the activity of the nitrifying bacteria in the aerobic culture is more pronounced than that of the de-nitrifying organisms under water-logged condition.

It is evident that there is approximately an inverse relationship between bacterial numbers and ammonia. An increase in the bacterial population is accompained by a decrease in ammonia and *vice versa*. This relationship obviously reflects decreased bacterial activity due to increase in numbers.

The results of nitrite and nitrate determinations are shown in Table VI.

TABLE VI

Showing nitrite and nitrate formation

(*a*) *Nitrite-nitrogen as parts per million*

Treatments		*Dorsa* soil									*Matasi* soil					
		Days from commencement									Days from commencement					
		0	7	14	21	28	35	42	49	56	0	7	14	21	28	35
Ammonium sulphate	AE	0·91	1·70	0·40	0·29	0·41	0·59	0·51	0·19	0·16	0·17	0·09	0·32	Slight	Slight	0·14
	WL	0·91	0·32	0·98	1·21	0·56	*Nil*	0·19	Slight	Slight	0·17	Slight	*Nil*	0·02	*Nil*	0·39
Farmyard manure	AE	0·43	0·37	0·33	0·37	0·41	0·58	0·87	0·23	0·11	Slight	0·61	0·11	0·13	0·14	0·10
	WL	0·43	0·36	0·42	0·34	0·23	0·34	0·41	0·15	Slight	Do.	*Nil*	0·09	0·14	0·19	0·67
No nitrogen	AE	0·76	0·17	0·36	0·51	0·46	0·32	0·44	0·31	0·19	0·17	0·75	0·31	0·15	0·39	0·31
	WL	0·76	0·40	0·50	0·39	0·68	0·31	*Nil*	0·30	Slight	0·17	*Nil*	Slight	0·17	0·18	0·31

AE = Aerobic.
WL = Water-logged.

(*b*) *Nitrate-nitrogen in parts per million*

Treatments		*Dorsa* soil									*Matasi* soil					
		Days from commencement									Days from commencement					
		0	7	14	21	28	35	42	49	56	0	7	14	21	28	35
Ammonium sulphate	AE	110·4	108·7	102·5	105·4	128·7	180·3	127·2	137·6	117·5	18·7	15·6	31·3	22·4	33·5	30·6
	WL	110·4	82·4	20·4	12·4	Slight	5·5	*Nil*	*Nil*	*Nil*	18·7	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
Farmyard manure	AE	96·0	92·6	119·6	81·7	118·9	90·1	93·2	88·8	109·4	19·0	30·6	23·4	61·5	40·9	36·5
	WL	96·0	106·5	19·8	11·1	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	19·0	*Nil*	*Nil*	*Nil*	*Nil*	2·9
No nitrogen	AE	92·0	90·6	88·9	72·0	82·8	105·1	111·1	102·7	92·7	17·1	21·8	25·1	39·7	30·6	33·4
	WL	92·0	56·9	38·8	16·8	39·5	Slight	5·9	6·8	Slight	17·1	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*

AE = Aerobic.
WL = Water-logged.

There was greater accumulation of nitrite in *Dorsa* than in *Matasi* soil. In either soil the quantity of nitrite in the aerobic cultures exceeded, on the average, that in the corresponding water-logged cultures. It may also be noted that either very little or no nitrate was formed in the submerged cultures.

Since nitrite cannot be detected in sufficient quantities in soils covered with water, it may be concluded that some gaseous nitrogen is given off as the result of the activity of the de-nitrifying organisms. It is probable that the predominance of these organisms in submerged soils serves a very useful purpose in promoting the supply of oxygen to the roots of the rice plant through the reduction of nitrate. Harrison and Aiyer [1913-14] also concluded that the oxygen evolved at the surface of the submerged paddy soil is utilised by the rice plant for the purpose of aeration of the roots. These authors, however, trace the evolution of oxygen to a film of organised growth spread over the surface of the soil. It is also interesting to observe that according to Barritt [1933] de-nitrification occurring in percolating sewage filters accelerates to a very marked extent the oxidation of the organic matter in the sewage.

Reduction of nitrate in submerged soils

The experiments described below were carried out in order to ascertain fully the fate of nitrate added to these soils and gain direct evidence regarding the loss of nitrate under water-logged condition.

Aerobic (moistened to fifty per cent of the water-holding capacity of the soil) and water-logged cultures, similar to those already described, were set up with the same samples of *Dorsa* and *Matasi* soils used in the previous experiments. To each sodium nitrate was added at the rate of 0·05 grm. nitrogen per kg. of dry soil. Soil samples were withdrawn daily and analysed for nitrate, nitrite and ammonia contents.

The daily changes in nitrate-nitrogen are shown in Table VII.

TABLE VII

Reduction in added nitrogen

Days from start	*Dorsa* soil		*Matasi* soil	
	Nitrate nitrogen as parts per million		Nitrate nitrogen as parts per million	
	Aerobic	Water-logged	Aerobic	Water-logged
0	95·00	95·00	67·05	67·05
1	..	..	80·87	34·10
2	90·03	76·01	80·62	49·20
3	89·90	64·25	79·04	50·50
4	104·15	47·34	73·60	48·80
5	96·60	47·62	76·49	49·30
6	96·34	39·30	73·80	49·50
7	103·30	35·17	73·80	29·30
8	102·60	28·04	72·02	42·60
9	95·70	22·50	64·09	31·20
10	88·09	22·70	61·42	25·70

It is obvious that a considerable portion of nitrate disappears very quickly on water-logging the soils—the rate of diminution being much greater in the heavier soil than in the lighter one. In neither of the soils was there any marked disappearance of nitrate under aerobic conditions during the first ten days ; whereas, during the same period under water-logged condition the losses registered by *Dorsa* and *Matasi* soils amounted, respectively, to as much as seventy-seven per cent and sixty-two per cent of the quantity originally added. It is therefore evident that the nitrate-reducing bacteria are more active in *Dorsa* than in *Matasi* soil.

The nitrite and ammonia figures are given below.

TABLE VIII

Showing accumulation of nitrite and ammoniacal nitrogen in soil receiving sodium nitrate

Days from start	*Dorsa* soil Nitrogen as parts per million				*Matasi* soil Nitrogen as parts per million			
	Aerobic		Water-logged		Aerobic		Water-logged	
	NO_2-N	NH_3-N	NO_2-N	NH_3-N	NO_2-N	NH_3-N	NO_2-N	NH_3-N
0 . .	0·76	52·0	0·76	52·0	0·17	48·0	0·17	84·0
1 . .	2·41	51·0	Slight	37·7	0·67	61·0	0·19	37·5
2 . .	1·92	40·2	Do.	35·8	0·29	58·6	0·17	39·2
3 . .	1·20	40·9	Do.	37·2	0·63	61·0	0·13	..
4 . .	1·20	49·2	Do.	42·5	0·31	59·7	0·10	42·8
5 . .	2·38	45·8	Do.	42·6	0·60	58·2	0·10	44·6
6 . .	4·04	39·0	Do.	37·0	0·61	61·3	0·10	46·5
7 . .	3·33	62·0	Do.	56·9	0·66	56·3	0·46	50·0
8 . .	0·88	25·3	Do.	24·7	0·37	48·7	0·19	51·0
9 . .	0·52	28·2	0·74	45·5	0·11	49·8	0·23	52·2
10 . .	0·59	34·5	0·90	44·0	0·10	53·0	0·17	43·9
11 . .	..	31·1	..	42·2	..	57·0	..	64·3
12 . .	..	34·2	..	44·4	..	66·7	..	52·3

In spite of the fluctuations in the figures it is evident that during the first few days less nitrite and ammonia were formed in the soils kept under water than in those under ordinary arable conditions. A similar reduction of nitrate, although not so marked, has already been observed in the water-logged cultures of these soils receiving farmyard manure, sulphate of ammonia or no nitrogen.

In both the soils the diminution of nitrate nitrogen under water-logged condition was much in excess of the nitrite and ammonia formed. The intense activity of de-nitrifying organisms in the submerged soils is vividly brought out by the fact that not only a very large portion of the added nitrate was reduced within a few days but also the ammonia and nitrite already present in the soil suffered reduction to a marked extent.

It may be seen that after the first week the accumulation of ammonia in the water-logged cultures exceeded that in the corresponding aerobic cultures, the difference being more pronounced in *Dorsa* than in *Matasi* soil. Probably this is due to the efficiency of the de-nitrifying bacteria diminishing after a time owing to increase in numbers as has been observed by one of the authors in relation to the effect of bacterial population on the production of carbon dioxide from soil.

Experiment in culture solutions

The following experiments were carried out for studying more effectively the comparative influence of the different treatments on the nitrate-reducing organisms in these soils :—

One hundred grm. portions of soils were treated individually with farmyard manure and sulphate of ammonia, each at the rate of 100 mg. of nitrogen per kg. of soil. These cultures along with those receiving no nitrogen were incubated for three weeks both under aerobic and water-logged conditions, and afterwards 100-c.c. lots of nutrient solutions in separate 250 c.c. Erlenmeyer flasks were inoculated with one grm. of soil (on dry basis) from each of the treatments. The nutrient solution was prepared by sterilizing separately and mixing equal quantities of the following :

(*a*) 2·5 grm. potassium nitrate per litre ;

(*b*) 2·0 grm. potassium phosphate, 0·4 grm. magnesium sulphate, 0·2 grm. each calcium chloride, potassium chloride and sodium chloride, and 0·004 grm. ferric chloride per litre.

The flasks were incubated at 32°C. and 2 c.c. of the solution withdrawn daily by sterilized pipettes for nitrate determination. The mean daily nitrate nitrogen for four-day intervals are given in Table IX.

Table IX

Nitrate-nitrogen in mg. per litre (means of four days)

Treatments		*Dorsa* soil				*Matasi* soil			
		Days			Mean difference in NO_3 (AE—WL)	Days			Mean difference in NO_3 (AE—WL)
		1-4	5-8	9-12		1-4	5-8	9-12	
		NO_3	NO_3	NO_3		NO_3	NO_3	NO_3	
No nitrogen	AE. .	323	300	323	+120	305	300	323	+20
	WL..	263	278	285		285	300	323	
Ammonium sulphate	AE. .	322	315	330	+143	293	338	341	+23
	WL..	240	292	292		285	345	319	
Farmyard manure	AE. .	293	330	313	+13	325	341	345	+118
	WL..	293	322	308		293	292	308	

AE = Aerobic.
WL = Water-logged.

The solution cultures inoculated with soils from the submerged treatments gave less nitrate than those inoculated with soils from the corresponding aerobic treatments. It is, therefore, evident that the de-nitrifying bacteria occurring in the water-logged soils functioned effectively after inoculation into solution cultures.

The mean difference in the amounts of nitrate in cultures inoculated, respectively, with soils incubated under aerobic and water-logged conditions forms for each treatment a measure of the activity of the nitrate-reducing organisms. Accordingly, it may be seen that in the presence of a mixed soil flora the de-nitrifying bacteria in the sulphate of ammonia and no nitrogen submerged treatments show greater vigour and activity in *Dorsa* than in *Matasi* soil ; with farmyard manure, on the contrary, de-nitrification is much more intense in the latter so ilthan in the former. It is rather striking that the lowering of the bacterial numbers as effected by these treatments in water-logged soil cultures also leads to similar conclusions.

The above data further help in understanding the relative influence of sulphate of ammonia and farmyard manure (as compared with no nitrogen treatment) on the de-nitrifying bacteria present in either of the soils under water-logged conditions. Sulphate of ammonia, while proving beneficial to the de-nitrifiers in *Dorsa* soil, does not seem to exert any marked influence on these orgamisms in *Matasi* soil. Farmyard manure, however, has a distinctly toxic effect on the de-nitrifying bacteria in *Dorsa* soil although it favours very appreciably their activity in *Matasi* soil.

Carbon dioxide production

Some of the changes taking place in the soil as a result of anaerobic decomposition of green manure under water-logged condition have been studied by Harrison and Aiyer [191 -14]. Methane, hydrogen and carbon dioxide are generally evolved in the body of the soil ; but at the surface the change is entirely different and the gases consist only of oxygen and nitrogen. The difference was attributed to a film of organisms which have the power of converting methane into carbon dioxide and this into oxygen. Söhngen [1906] first isolated from the soil *B. methanicus* which could transform methane partly into organic compounds and partly into carbon dioxide. Certain other common soil bacteria, capable of converting methane into carbon dioxide under anaerobic conditions have since been discovered. Münz [1915] has shown that some bacteria use the energy obtained by the oxidation of methane for chemeosynthetic utilization of carbon dioxide. This process may be represented by the equation $CH_4 + 2O_2 = CO_2 + 2\ H_2O +$ 218 Cals.

It was accordingly thought of interest to follow the evolution of free carbon dioxide under water-logged condition from the two rice soils, *Matasi* and *Dorsa*, each receiving farmyard manure, sulphate of ammonia and no nitrogen. For the sake of comparison the carbon dioxide produced from each of these soils under aerobic condition was also determined.

Thetotl carbon dioxide produced in successive intervals of twenty-four hours was determined by aspirating air at the rate of four to five litres per day over 250 grms. of soil in 1000-c.c. Erlenmeyer flasks and absorbing it in a known volume of a standard baryta solution in Pettenkoffer tubes. The excess of baryta left in the tubes was determined by titration against standard hydrochloric acid. For each individual treatment two similar flasks were set up—one containing submerged soil and the other containing soil moistened to fifty per cent of its water-holding capacity. The doses of farmyard manure and sulphate of ammonia were each at the rate of 100 mg. nitrogen per kg. of soil.

The mean daily carbon dioxide production for four-day periods is given in Table X.

TABLE X

Carbon dioxide production (means of four days)

Treatments		CO_2 evolution. Daily rate in mg. per kg. of soil. Days			Total CO_2 produced in 12 or 13 days	Ratio of CO_2 evolution AE : WL
		1-4	5-8	9–12		
			(*a*) *Matasi soil*			
No nitrogen	AE. .	67·6	55·3	43·5	665·6	1·90
	WL..	24·2	25·8	37·6	350·2	
Ammonium sulphate.	AE. .	35·4	34·2	44·9	458·0	1·03
	WL..	60·0	28·1	23·6	446·7	
Farmyard manure.	AE. .	120·7	78·5	30·7	919·2	1·14
	WL..	86·4	92·5	23·6	809·7	
			(*b*) *Dorsa soil*			
No nitrogen	AE. .	97·9	69·3	61·0	912·7	1·36
	WL..	54·8	52·1	61·5	673·2	
Ammonium sulphate.	AE. .	82·9	38·1	39·8	643·2	1·83
	WL..	23·2	23·6	41·1	351·3	
Farmyard manure.	AE. .	75·0	73·5	20·3	675·2	1·48
	WL..	47·4	53·5	12·9	455·1	

AE = Aerobic.
WL = Water-logged.

It will be seen that in each case more carbon dioxide was evolved from the aerobic culture than from the corresponding water-logged one. From the ratios of carbon dioxide evolution between aerbic and submerged soils (given in Table X) it is, however, obvious that the production of carbon dioxide under water-logged condition did not lag much behind that under aerobic condition.

In the present set of experiments the surfaces of the submerged soils were carefully examined from time to time, but the formation of an algal film could not be traced in any of the cultures. Moreover, the presence of a film of micro-organisms on the surface would have almost completely prevented the evolution of free carbon dioxide by converting it into oxygen.

It has already been pointed out that the numbers of the strictly aerobic bacteria in the submerged cultures of both the soils, although much lower than those in the aerobic cultures, are sufficiently large to prove the presence of a fairly abundant supply of oxygen in the water-logged soils. It seems therefore reasonable to assume that as far as these soils are concerned the processes leading to the formation of carbon dioxide under submerged and aerobic conditions are more or less the same. The diminution in the production of carbon dioxide as a result of water-logging is according to expectation, since the supply of oxygen is less in the submerged culture than in the corresponding aerobic one.

The relative effect of water-logging on the carbon dioxide evolution is, however, not the same in the two soils. In the *Matasi* soil water-logging diminishes to a much greater extent the production of carbon dioxide in the untreated culture than in either of the treated cultures. The reverse is, however, the case in the *Dorsa* soil in which water-logging causes an appreciably greater diminution in the evolution of carbon dioxide in the treated cultures than in the control. Both under aerobic and submerged conditions more carbon dioxide was produced from *Dorsa* than from *Matasi*. With farmyard manure, on the contrary, more carbon dioxide was liberated from *Matasi* soil than from *Dorsa*. It is also evident that in the latter soil sulphate of ammonia inhibits carbon dioxide production to a very marked extent. It is interesting to point out here that the bacterial numbers, as obtained by plate counts, are higher in *Dorsa* than in *Matasi* soil. Also sulphate of ammonia distinctly lowers the bacterial numbers in *Matasi* soil.

Whatever the exact mechanism leading to the formation of carbon dioxide in water-logged soils, the results of the experiments summarised above tend to show clearly that there is a close similarity in the processes by which carbon dioxide is produced in these soils under aerobic and water-logged conditions.

Summary

The biochemical processes involved in the decomposition of farmyard manure and sulphate of ammonia under water-logged and aerobic conditions in two of the main rice soils of the Central Provinces were investigated in the laboratory.

The numbers of the strictly aerobic bacteria in the submerged cultures of both the soils, although much lower than those in the aerobic cultures, were sufficiently large to prove the presence of a fairly abundant supply of oxygen in the rice soils during the crop season.

Application of either farmyard manure or sulphate of ammonia depresses the population (both under aerobic and water-logged conditions) in the heavier soil, *Dorsa*, while no such effect was noticed in the lighter soil, *Matasi*.

In *Matasi* soil water-logging in general led to a diminution in ammonia. In *Dorsa* soil there was, as a result of water-logging, no change in ammonia in cultures receiving farmyard manure, but the one which contained sulphate of ammonia showed a significant increase.

An inverse relationship exists between the bacterial numbers and ammonia accumulation.

The accumulation of nitrite was more in the heavier soil than in the lighter one. Nitrate was almost entirely absent in the submerged cultures of either soil.

In one of the soils (*Dorsa*) sulphate of ammonia proved beneficial and farmyard manure toxic to the de-nitrifying bacteria. But in the other soil (*Matasi*) while sulphate of ammonia did not show any effect on these organisms, farmyard manure distinctly favoured their activity.

The amount of carbon dioxide produced from each of these soils was measured. The results indicate that the processes leading to the formation of carbon dioxide under submerged and aerobic conditions are essentially the same.

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STATISTICAL NOTES FOR AGRICULTURAL WORKERS

NO. 16—A COMPLEX EXPERIMENT ON RICE AT THE CHINSURAH FARM, BENGAL, 1933-34

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Introduction

A large number of agricultural experiments are designed to elucidate information only on simple and direct issues, as for example, the relative efficiency of different doses of nitrogenous manures, or the yielding capacities of certain selected strains of rice. It is however often desirable to study the effect of one factor in relation to a second. For example, the agriculturist may be interested to know the individual requirements of doses of manures, etc., for his different varieties of rice; for it is well known that all varieties do not respond equally to the same manurial or cultural treatment. For studying such interactions between two or more factors affecting yields of crops, the most suitable technique is the 'Complex Experiment'. In such experiments, the relevant factors are superimposed and laid out in one large but compact plot design, with suitable block divisions for convenience of agricultural operations and elimination of soil heterogeneity. It has been found that the complex experiment "by allowing every one of a set of differential treatments to be combined with every one of another set, gives to each set of comparisons the same precision as if the whole experiment had been devoted to it alone. In addition, the design provides information on the same level of precision upon any differential responses that

may exist among the different treatments ". Finally, the large amount of data available in a complex experiment allow, after elimination of soil differences and treatment effects, sufficient degrees of freedom for a precise estimate of the residual error.

Object of the experiment

A study of response of rice plants to cultural treatments is for many reasons a very important problem for Bengal. In fact, for many parts of this province, a manurial treatment of rice fields is not essential, and is also not likely to be of much economic significance. What is particularly needed is to increase yield by a careful selection of heavy-yielding varieties suitable for each district and situation, and a detailed study of the effect of cultural operations such as spacing, water requirements at various stages of plant growth, time of planting and seedling number per hole, etc., on the yield per acre. Of these, the water requirement plays a very important part at different stages of the plant life and determines to a large extent the final yield; but for want of proper facilities, this factor could not be included in the present test. Three other factors, namely, time of planting, spacing, and seedling number per hole were combined in one complex experiment, and their effects on three varieties of rice with different ripening habits have been studied in all possible combinations.

Method of experiment

With a view to examine these mutual interactions, a four-fold complex experiment was designed in the Statistical Laboratory, Calcutta, and was laid out in the Chinsurah Farm, Bengal, in 1933, and comprised the following four sets of factors :—

(I) Three different varieties of rice :—

(1) *Nehara* :—with early ripening habit.
(2) *Bhasamanik* :—with medium ripening habit.
(3) *Bhasakalma* :—with late ripening habit.

(II) Five dates of planting :—

(1) July 16.
(2) August 1.
(3) August 16.
(4) September 1.
(5) September 16.

(III) Three spacings :—

(1) Six inches.

(2) Nine inches.

(3) Twelve inches.

(IV) Three different numbers of seedlings per hole :—

(1) 1 plant per hole.

(2) 2 plants per hole.

(3) Local method (2 to 4 plants per hole).

Taking all the four variates, there were ($3\times5\times3\times3$) or 135 different treatments. Each treatment was replicated three times, giving a total of 405 plots. The total area under the experiment was three acres, and the area per unit plot was 9 ft.$\times$ 18 ft. (1/269 acre approximately).

The lay-out adopted is shown in the annexed diagram. The experiment was divided into three blocks, each of which contained one set of all treatments. The plots of the same sowing date within each block were grouped together, and the position occupied by the sowing date groups within the blocks were determined at random. This grouping together of plots of the same sowing date was adopted to facilitate cultural operations. For the same reason, the three varieties were also laid out in compact rows. The nine combinations of spacings and seedling numbers were then thrown at random within each combination of date of planting and variety as shown in the diagram. Actual yields are given in the appendix.

	Sept. 1	July 16	Aug. 1	Sept. 16	Aug. 16
Nehara	D	A	D	E	I
	F	F	H	B	A
	E	B	I	F	H
Bhasa-manik	G	G	E	C	D
	A	E	A	I	F
	I	I	F	G	G
Bhasa-kalma	B	C	G	D	C
	C	H	C	H	B
	H	D	B	A	E
Bhasa-kalma	F	A	E	B	G
	G	I	F	C	A
	E	G	A	F	H
Nehara	I	H	C	G	D
	C	E	B	I	F
	A	D	G	A	B
Bhasa-manik	D	F	H	D	E
	H	C	D	E	I
	B	B	I	H	C
Bhasa-kalma	I	F	I	I	I
	D	B	H	E	D
	A	E	D	G	F
Bhasa-manik	E	A	G	B	B
	F	H	B	F	A
	C	D	C	A	H
Nehara	G	G	E	D	C
	H	I	A	H	G
	B	C	F	C	E

	Aug. 1	Aug. 16	Sept. 1	Sept. 16	July 16
Bhasa-kalma	D	D	C	E	E
	I	G	A	G	I
	E	C	H	C	G
Bhasa-manik	B	E	F	F	H
	G	F	I	D	A
	A	I	D	I	F
Nehara	F	H	G	H	B
	C	A	B	A	D
	H	B	E	B	C
Bhasa-kalma	B	I	I	B	B
	A	E	G	F	D
	F	F	F	D	F
Nehara	I	D	C	C	G
	D	C	A	I	A
	G	G	D	E	H
Bhasa-manik	C	A	E	H	E
	H	H	B	A	I
	E	B	H	G	C
Nehara	E	E	H	G	F
	B	F	I	F	I
	A	I	F	D	E
Bhasa-manik	D	D	C	E	D
	I	G	G	B	G
	F	C	A	C	B
Bhasa-kalma	C	B	E	H	C
	G	A	B	A	A
	H	H	D	I	H

	Sept. 16	Aug. 16	Sept. 1	Aug. 1	July 16
Bhasa-kalma	E	C	E	I	A
	I	H	C	E	G
	F	A	F	F	C
Bhasa-manik	B	I	I	B	F
	H	D	A	H	I
	G	G	H	G	H
Nehara	D	E	G	A	D
	C	B	D	C	B
	A	F	B	D	E
Bhasaa kalma	B	F	A	C	H
	G	E	H	A	B
	H	I	B	D	E
Nehara	F	G	I	H	F
	I	C	E	G	G
	E	D	F	B	I
Bhasa-manik	D	B	D	E	D
	A	H	C	I	A
	C	A	G	F	C
Nehara	G	H	A	F	H
	H	A	H	I	A
	B	I	C	E	C
Bhasa-manik	F	F	F	A	E
	E	C	B	C	B
	B	E	E	D	G
Bhasa-kalma	D	B	G	G	D
	C	D	D	H	I
	A	G	I	B	F

Different numbers of seedling per hole .	Spacing 6″	9″	12″
(1) 1 Plant per hole	A	B	C
(2) 2 Plants per hole	D	E	F
(3) Local methods (2 to 4 plants per hole) .	G	H	I

Diagram showing plan of the experiment.

The harvest was gathered between 18th November and 21st December 1933. Besides the yield of grain and straw, the following characters were also observed :—

(1) Date of flowering in the different plots.
(2) Height of the plants in the different plots.
(3) Length of inflorescence in the different plots.
(4) Number of tillers in the different plots.

Rainfall

The weekly totals of rainfall in the Chinsurah Farm during the season together with the number of rainy days in the week are given in Table I. It will be seen that the rainfall was well-distributed over April and May ; the downpour however began to be excessive in June. The normal for the month is 10·56 in., whereas the actual rain was 17·65 in. Besides, 7·28 in. of rain in one day (24th June), flooded the entire farm and there was great difficulty in draining out the water. Normal rain continued throughout July, August and September. The result was that transplanting had to be done under very unfavourable circumstances ; and the standing water, which persisted to a depth of more than one foot, affected the varieties considerably, and the tillering was uniformly poor. The young seedlings thus did not get a good start, and the final yields were rather poor.

Table I

Weekly totals of rainfall in inches at Chinsurah Farm, 1933

Period	Total rain-fall	No. of rainy days	Period	Total rain-fall	No. of rainy days
March 1—31 .	0·00	0	July 8—14 .	1·35	5
			,, 15—21 .	3·12	5
April 1—7 .	0·00	0	,, 22—28 .	1·60	7
,, 8—14 .	2·38	3	,, 29—4 Aug.	1·34	4
,, 15—21 .	0·97	1			
,, 22—28 .	1·50	2	Aug. 5—11 .	2·68	6
,, 28—5 May	1·53	3	,, 12—18 .	4·80	7
			,, 19—25 .	6·03	7
May 6—12 .	0·88	4	,, 26—1 Sept.	1·77	5
,, 13—19 .	0·54	3			
,, 20—26 .	0·37	1	Sept. 2—8 .	2·07	4
,, 27—2 June	1·96	5	,, 9—15 .	0·39	3
			,, 16—22 .	6·98	5
June 3—9 .	2·38	2	,, 23—29 .	1·18	4
,, 10—16 .	1·81	5	,, 30—6 Oct.	1·54	1
,, 17—23 .	5·01	7			
,, 24—30 .	8·12	5	Oct. 7—13 .	0·00	0
			,, 14—20 .	2·56	5
			,, 21—27 .	0·67	2
July 1—7 .	4·25	6	,, 28—3 Nov.	0·00	0

STATISTICAL ANALYSIS OF DATA AND DISCUSSION OF RESULTS

The analysis of variance† of the yield of grain is shown in Table II. The actual yield data are given in the appendix.

TABLE II

Analysis of variance, yield of grain in tolas

Factor of variation	Degrees of freedom	Sum of squares	Variance	Mean variance / Error variance
Block	2	288,195	144,097·50	..
Date of planting	4	9,559,367	2,389,841·75	78·45**
Error	8	243,685	30,460·63	..
Variety	2	1,417,033	708,516·50	120·12**
Planting × Variety	8	236,867	29,608·37	5·01**
Error	20	117,966	5,898·30	..
Spacing	2	129,695	64,847·50	47·54**
Seedling	2	65,381	32,690·50	23·96**
Seedling × Spacing	4	5,583	1,395·75	1·02
Planting × Seedling	8	19,871	2,483·87	1·82
Planting × Spacing	8	42,563	5,320·37	3·90*
Varieties × Seedling	4	4,450	1,112·50	0·81
Varieties × Spacing	4	5,226	1,306·50	0·95
Planting × Variety × Seedling	16	63,854	3,990·87	2·92*
Planting × Variety × Spacing	16	47,239	2,952·43	2·16*
Planting × Seedling × Spacing	16	23,070	1,441·87	1·05
Variety × Seedling × Spacing	8	9,586	1,198·25	0·87
Planting × Variety × Seedling × Spacing.	32	30,295	946·71	0·69
Error	239	326,007	1,364·05	..

N.B.—Items marked with two stars (**) are significant on a level of one per cent probability, and those marked with a single star (*) on the level of five per cent probability.

†The yield of one plot was found missing. This was reconstructed by the method of minimising the error variances described by Yates in "the Analysis of Replication Experiments when the Field Results are Incomplete", *Emp. J. Expt. Agric., Vol.* **1**, 1930, 129-142.

From Table II the significant effects can at once be located—

(I) Among primary effects—

(1) the date of planting.

(2) variety.

(3) spacing, and

(4) number of seedlings

are all significant.

(II) First-order interactions between—

(5) date of planting and variety, and

(6) date of planting and spacing

are significant.

(III) Second-order interactions between—

(7) date of planting, variety, and number of seedling,

(8) date of planting, variety, and spacing

are significant.

(IV) The third-order interaction is inappreciable.

These results may now be shown quantitatively. The primary results are shown in Table III.

TABLE III

Mean yield of grain in tolas per plot

Varieties	Mean yield	Date of planting	Mean yield	Spacing	Mean yield	No. of seed-lings	Mean yield
Nehara	278.62	July 16	434.27	6 in.	374.03	1	337.25
Bhasamanik . . .	423.10	Aug. 1	477.99	9 in.	357.41	2	356.77
Bhasakalma	360.31	Aug. 16	438.75	12 in.	330.59	Local	368.00
		Sept. 1	363.25				
		Sept. 16	55.80				
Mean	354.01		354.01		354.01		354.01
Standard error . . .	2.20		4.22		3.17		3.17

Variety.—The medium ripening *Bhasamanik* is by far the largest yielder, then comes the late variety *Bhasakalma*, while the early *Nehara* gives the lowest yield. The differences are considerable in magnitude.

Date of planting.—The highest yield was obtained for the second date of planting (August 1); the first (July 16) and the third (August 16) are practically identical, while the fourth (September 1) is decidedly inferior to the first three, and the last one (September 16) is a clear failure.

Spacing.—The closest spacing (6 in.) gives the highest yield, nine in. spacing gives four per cent lower yield, and twelve in. spacing twelve per cent lower yield than the six in. spacing.

Number of seedlings per hole.—The local method (two to four seedlings) gives the best results ; yields with one or two seedlings are significantly lower.

Interactions.—In order to appreciate the full implications of the primary effects, an examination of the significant interactions is essential.

The interactions between " variety " and " date of planting " are shown in Table IV.

TABLE IV

Interaction of date of planting and variety (mean yield of grain in tolas per plot)

Variety	Date of planting					Mean
	July 16	Aug. 1	Aug. 16	Sept. 1	Sept. 16	
Nehara	372.81	396.90	357.29	260.35	5.74	278.62
Bhasamanik . . .	474.68	541.18	498.68	439.72	161.22	423.10
Bhasakalma	455.31	495.88	460.29	389.68	0.40	360.31
Mean	434.27	477.99	438.75	363.25	55.80	...
	Standard error = 4.93					
Difference between Varieties—						
Bhasamanik—Bhasakalma .	19.37	45.30	38.39	50.04	160.82	...
Bhasakalma—Nehara .	82.50	98.98	103.00	129.33	—5.34	...
Bhasamanik—Nehara .	101.87	144.28	141.39	179.37	155.48	...
	Standard error of difference = 6.97					

It will be seen that all the differences except one are statistically significant. Ignoring the 5th planting (September 16) for the present, since for both *Nehara* and *Bhasakalma*, it was a failure, it is evident that the 2nd (August 1), 3rd (August 16), and 4th dates (September 1) give much larger differences compared to the first (July 16).

The difference between *Bhasamanik* and *Bhasakalma* is thus less marked for July 16 than for August 1, August 16 or September 1. Similarly, the difference between *Bhasakalma* and *Nehara* is more marked for August 16 and September 1 than for July 16 or August 1. As regards *Bhasamanik* and *Nehara*, the difference is more marked for August 1 and August 16, and particularly for September 1 than for July 16.

The interactions between " date of planting " and " spacing " are given in Table V.

TABLE V

Interaction of date of planting and spacing (mean yield of grain in tolas per plot)

Spacing	Date of planting					Mean
	July 16	Aug. 1	Aug. 16	Sept. 1	Sept. 16	
(6 in.)	453.35	479.35	459.20	401.57	76.68	374.03
(9 in.)	437.61	486.48	438.74	371.83	52.39	357.41
(12 in.)	411.85	468.14	418.33	316.35	38.29	330.59
Mean	434.27	477.99	438.75	363.25	55.80	354.01
	Standard error = 7.17					
Differences—						
(6 in.)—(9 in.)	15.74	—7.13	20.46	29.74	24.29	...
(9 in.)—(12 in.) . . .	25.76	18.34	20.41	55.48	14.10	...
(6 in.)—(12 in.) . . .	41.50	11.21	40.87	85.22	38.39	...
	Standard error of difference = 10.10					

A spacing of six in. is practically of no advantage over nine in. for the first two dates of planting. Its usefulness increases very rapidly as the date of planting is more and more delayed.

Between nine in. and twelve in., the difference was always significantly in favour of the nine in., except for the second date, when the difference was not appreciable. Here also a closer spacing is found to be more useful for the late planting.

Between six in. and twelve in., the differences are considerable, but still for the second date of planting the difference is insignificant.

We thus find that neglecting the 5th date of planting (September 16) which was a failure in certain cases :—

(1) A closer spacing is extremely useful if the date of planting is delayed.

(2) For the normal date of planting in this part of Bengal (August 1), the yields of grain for all the three spacings did not differ appreciably. The conclusion cannot however be considered final, since in this particular year the first date of planting was prejudicially affected by heavy rain.

(3) For the early planting (July 16), the difference between nine in. and twelve in., also between six in. and twelve in. is significant.

The second-order interactions between "variety", "date of planting", and "spacing" are given in Table VI, and the various differences in Tables VII and VIII.

Table VI

Interaction of variety, planting and spacing (mean yield of grain in tolas per plot)

Variety	Spacing	Date of planting					Mean
		July 16	Aug. 1	Aug. 16	Sept. 1	Sept. 16	
Nehara	6 in.	386.8	396.0	387.5	298.8	7.8	295.38
	9 in.	376.1	406.2	347.3	280.4	5.7	283.14
	12 in.	355.6	388.6	337.1	201.8	3.8	257.22
Bhasamanik	6 in.	497.0	549.1	513.4	469.2	221.9	450.12
	9 in.	480.8	541.0	500.6	436.8	151.4	422.12
	12 in.	446.4	533.4	482.0	413.1	110.4	397.06
Bhasakalma	6 in.	476.4	492.9	476.6	436.7	0.4	376.60
	9 in.	455.9	512.3	468.3	398.2	0.1	366.96
	12 in.	433.6	482.4	436.0	334.2	0.7	337.38
Mean	...	434.27	477.99	438.75	363.25	55.80	354.01

Standard error = 12.31

Failure of *Nehara* and *Bhasakalma* on September 16 is evident from the last column.

Table VII

Mean difference in yield (in tolas per plot) between 6 in. and 9-in. spacing

Varieties	Date of planting				
	July 16	Aug. 1	Aug. 16	Sept. 1	Sept. 16
Nehara	10.7	—10.2	40.2	18.4	2.1
Bhasamanik	16.2	8.1	12.8	32.4	70.5
Bhasakalma	20.5	—19.4	8.3	38.5	0.3
Mean	15.80	—7.17	20.44	29.77	24.30

Standard error of difference = 17.35

Table VII shows that differences between six in. and nine in. are insignificant for July 16 and August 1 for all the varieties.

In Table V the superiority of six in. over nine in. was significant for August 16; this because *Nehara* (early variety) alone showed this tendency while for the two other varieties the differences were insignificant.

For September 1, both *Bhasamanik* and *Bhasakalma* gave better yields for six in. spacing than for nine in. For September 16, *Bhasamanik* alone gave an appreciable yield which certainly shows its remarkable adaptability.

TABLE VIII

Mean difference in yield (in tolas per plot) between 9-in. and 12-in. spacing

Varieties	Date of planting				
	July 16	Aug. 1	Aug. 16	Sept. 1	Sept. 16
Nehara	20.5	17.6	10.2	78.6	1.9
Bhasamanik	34.4	7.6	18.6	23.7	41.0
Bhasakalma . . .	22.3	29.9	32.3	64.0	—0.6
Mean	25.73	18.33	20.36	55.43	14.10

Standard error of difference = 17.35

Table VIII indicates for July 16 a big difference for *Bhasamanik*, while the two others show no appreciable difference as the spacing is changed from nine in. to twelve in. This significant increase of *Bhasamanik* is responsible for the positive response of July 16 in the first-order interaction.

For August 16, the difference for *Bhasakalma* alone is on the verge of significance. For September 1 both *Nehara* and *Bhasakalma* show considerable increase, while *Bhasamanik* gives practically the same yield for both spacings.

For September 16, *Nehara* and *Bhasakalma* figures are unreliable; but *Bhasamanik* shows a definite increase in yield for nine in. spacing against twelve in.

Table IX gives the second-order interactions between "date of planting", "variety", and "seedlings per hole", actual differences being given in Tables X and XI.

Table IX

Interaction of planting, variety and seedlings

Variety	Number of seedlings per hole	Date of planting					Mean
		July 16	Aug. 1	Aug. 16	Sept. 1	Sept. 16	
Nehara	1	381.4	378.5	343.6	228.0	3.8	267.06
	2	355.6	430.2	358.4	255.7	6.4	281.26
	Local method	381.4	382.0	369.8	297.4	7.1	287.54
Bhasamanik	1	463.6	538.1	489.3	414.6	102.4	401.60
	2	479.7	539.8	497.3	440.7	171.5	425.80
	Local method	480.8	545.7	509.5	463.8	209.8	441.92
Bhasakalma	1	436.2	487.2	418.6	372.9	0.6	343.10
	2	469.1	487.6	477.6	381.8	0.3	363.28
	Local method	460.6	512.8	484.7	414.3	0.3	374.54
Mean	...	434.27	477.99	438.75	363.25	55.80	354.01

Standard error = 12.31

Table X

Mean difference in yield (in tolas per plot) between one and two seedlings per hole

Varieties	Date of planting				
	July 16	Aug. 1	Aug. 16	Sept. 1	Sept. 16
Nehara	—25.8	51.7	14.8	27.7	2.6
Bhasamanik	16.1	1.7	8.0	26.1	69.1
Bhasakalma	32.9	0.4	59.0	8.9	—0.3

Standard error = 17.35

For July 16, all the differences are insignificant. For August 1, *Nehara* alone gives significant increase for two seedlings. For August 16, the differences for *Nehara* and *Bhasamanik* are not significant ; but it is significant for *Bhasakalma*. It may be noted that *Nehara* gives a maximum yield at this date.

For September 1, all the differences are insignificant. For September 16, *Bhasamanik* shows a definite increase for two seedlings over one seedling per hole.

TABLE XI

Mean difference in yield (in tolas per plot) between two seedlings per hole and local method

Varieties	Date of planting				
	July 16	Aug. 1	Aug. 16	Sept. 1	Sept. 16
Nehara . .	25·8	—48·2	11·4	41·7	0·7
Bhasamanik .	1·1	5·9	12·2	23·1	38·3
Bhasakalma .	−8·5	25·2	7·1	32·5	0·0

Standard error = 17·35

For all dates of planting the ' local method ' is as good as the two-seedling method for both *Bhasakalma* and *Bhasamanik* ; the latter however shows an increased yield under the ' local method ' only for September 16.

Nehara has given an anomalous result. For July 16, August 16, and September 16, the differences are insignificant ; for September 1, the difference is significantly in favour of ' local method ', while for August 1, the two-seedling method has given an appreciably higher yield than the local one. This particular behaviour for August 1 requires further investigation, before it can be considered as established.

All other interactions are insignificant.

It must be added that the results discussed above were almost certainly affected by the conditions of the season, and it is not unlikely that at least some of them will be modified under varying seasonal conditions. With a view to obtain information on this seasonal influence, the experiment is being repeated for three consecutive years, and the results will be discussed in subsequent notes.

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Summary

The present paper gives the results of a four-fold complex experiment on rice with three varieties with different ripening habits (*Nehara*, *Bhasamanik*, *Bhasakalma*) ; five dates of planting (July 16, August 1 and 16, September 1 and 16); three spacings (6-in., 9-in., 12-in). : and three different numbers of seedlings per hole (one plant, two plants, and local method) laid out in orthogonal and randomized plots in three blocks.

(1) All the three varieties of rice give the largest yield when planted on August 1. For this date of planting, the three different spacings, (6-in.), (9-in.) and (12-in.), make no appreciable difference in the total yield, although the total number of plants per plot in the three cases is in the proportion of 6 : 4 : 3. Again, the yield is not significantly different whether the method of planting is of ' local type ' or one seedling, or two seedlings per hole, except for *Nehara* (early ripening) which shows the highest yield for two seedlings per hole.

(2) The system of spacing and seedling-number per hole becomes increasingly important as the date of planting is delayed. Thus a closer spacing as well as a larger number of seedlings are necessary for later dates of planting. This is to be expected from physiological considerations of plant growth, but the quantitative results obtained in the present experiment have indicated the remarkable adaptability of *Bhasamanik* (medium ripening) for all the dates of planting and spacings. Even for the latest date of planting (September 16) it gave fairly large yield with all spacings and seedling numbers. *Bhasakalma* (late) and particularly *Nehara* (early) lack this important quality, and need closer spacing and greater seedling number for maintaining a high yield. Both of them failed for September 16 under all conditions of spacing and seedling number.

Appendix

YIELDS OF RICE

Field 1

	September 1		July 16		August 1		September 16		August 16	
Nehara	D	264·0	A	376·0	D	381·5	E	10·0	I	308·5
	F	211·0	F	329·0	H	337·5	B	3·0	A	316·5
	E	296·0	B	338·0	I	344·0	F	2·0	H	322·5
Bhasa-manik	G	450·0	G	405·0	E	451·0	C	35·0	D	415·5
	A	416·0	E	418·5	A	475·0	I	141·0	F	400·5
	I	433·0	I	394·5	F	460·5	G	314·0	G	419·0
Bhasa-kalma	B	367·5	C	393·0	G	421·0	D	0·5	C	357·5
	C	243·0	H	406·0	C	397·5	H	*nil*	B	411·0
	H	398·5	D	406·0	B	423·5	A	*nil*	E	430·5
Bhasa-kalma	F	318·5	A	388·5	E	412·5	B	*nil*	G	451·5
	G	450·5	I	365·5	F	405·5	C	1·0	A	409·5
	E	374·0	G	401·5	A	404·5	F	*nil*	H	393·5
Nehara	I	207·0	H	333·0	C	301·5	G	16·5	D	313·0
	C	163·5	E	308·0	B	344·5	I	2·5	F	268·0
	A	225·5	D	306·5	G	340·0	A	8·0	B	298·5
Bhasa-manik	D	413·5	F	339·0	H	466·5	D	231·0	E	404·5
	H	439·0	C	370·5	D	448·5	E	173·0	I	403·5
	B	389·0	B	393·0	I	444·0	H	229·0	E	370·0
Bhasa-kalma	I	373·0	F	355·0	I	420·0	I	1.5	I	382·5
	D	425·5	B	377·0	H	455·5	E	*nil*	D	440·5
	A	413·0	E	372·5	D	380·5	G	*nil*	F	375·5
Bhasa-manik	E	457·0	A	382·0	G	464·0	B	120·5	B	452·0
	F	394·0	H	405·0	B	479·5	F	127·0	A	482·0
	C	351·0	D	448·0	C	462·5	A	174·0	H	462·0
Nehara	G	380·5	G	376·0	E	420·0	D	8·5	C	326·0
	H	381·5	I	327·0	A	415·5	H	5·0	G	413·0
	B	318·5	C	355·5	F	431·0	C	3·0	E	350·0

Field 2

	August 1		August 16		September 1		September 16		July 16	
Bhasa-kalma	D	573·0	D	612·5	C	375·0	E	1·0	E	551·5
	I	556·0	G	595·5	A	499·0	G	1·5	I	465·0
	E	585·5	C	509·0	H	496·0	C	3·0	G	519·0
Bhasa-manik	B	580·0	E	558·0	F	468·0	F	230·0	H	550·0
	G	577·0	F	570·0	I	492·0	D	416·0	A	542·0
	A	598·5	I	553·5	D	550·0	I	174·0	F	523·0
Nehara	F	417·0	H	375·5	G	295·0	H	4·0	B	417·0
	C	363·0	A	405·5	B	222·0	A	4·0	D	375·0
	H	418·5	B	292·5	E	278·0	B	5·0	C	352·0
Bhasa-kalma	B	518·0	I	523·0	I	333·5	B	*nil*	B	437·0
	A	579·0	E	576·5	G	488·0	F	1·0	D	516·5
	F	512·0	F	501·0	F	292·0	D	*nil*	F	406·5
Nehara	I	422·5	D	464·0	C	171·0	C	1·0	G	393·5
	D	460·5	C	364·5	A	337·5	I	4·0	A	378·5
	G	394·5	G	417·0	D	294·0	E	5·0	H	391·0
Bhasa-manik	C	549·0	A	586·5	E	410·0	H	193·0	E	473·5
	H	622·0	H	572·0	B	444·5	A	205·0	I	455·5
	E	594·0	B	529·0	H	506·0	G	258·5	C	395·0
Nehara	E	452·0	E	386·0	H	297·0	G	2·5	F	331·5
	B	405·0	F	345·0	I	312·5	F	4·0	I	325·0
	A	367·0	I	347·0	F	218·0	D	9·0	E	353·0
Bhasa-manik	D	602·0	D	548·0	C	399·0	E	182·0	D	451·0
	I	600·0	G	534·0	G	479·5	B	153·5	G	483·0
	F	571·0	C	507·0	A	469·0	C	100·0	B	416·0
Bhasa-kalma	C	540·0	B	503·5	E	349·5	H	*nil*	C	384·0
	G	548·0	A	312·5	B	360·5	A	1·5	A	445·0
	H	594·0	H	573·5	D	471·0	I	*nil*	H	477·0

Field 3

	September 16		August 16		September 1		August 1		July 16	
Bhasa-kalma	E	*nil*	C	394·0	E	462·0	I	580·5	A	547·0
	I	*nil*	H	486·0	C	355·0	E	551·0	G	518·0
	F	*nil*	A	517·0	F	361·0	F	481·5	C	456·0
Bhasa-manik	B	118·0	I	598·0	I	460·0	B	549·5	F	512·0
	H	191·0	D	616·0	A	501·0	H	554·5	I	527·0
	G	211·5	G	525·5	H	429·0	G	608·5	H	484·0
Nehara	D	3·5	E	386·0	G	274·0	A	429·0	D	408·0
	C	2·0	B	329·5	D	302·0	C	372·5	F	391·0
	A	3·0	F	330·5	B	176·0	D	430·0	E	408·5
Bhasa-kalma	B	*nil*	F	422·5	A	364·5	C	449·0	H	496·
	G	*nil*	E	487·5	H	397·0	A	521·0	B	499·0
	H	*nil*	I	458·0	B	379·0	D	486·0	E	487·0
Nehara	F	10·0	G	392·0	I	247·0	H	427·5	F	381·0
	I	5·5	C	376·5	E	273·0	G	346·0	G	434·5
	E	5·5	D	383·5	F	165·5	B	409·0	I	407·5
Bhasa-manik	D	173·5	B	502·0	D	458·0	E	572·0	D	561·0
	A	13·5	H	518·0	C	375·0	I	575·0	A	577·0
	C	2·0	A	494·5	G	486·0	F	573·0	C	501·0
Nehara	G	15·0	H	385·0	A	317·0	F	438·0	H	445·5
	H	8·5	A	383·0	H	282·0	I	407·5	A	433·0
	B	5·0	I	368·0	C	120·5	E	441·5	C	391·5
Bhasa manik	F	8·5	F	455·0	F	346·0	A	582·5	E	591·0
	E	2·5	C	480·5	B	387·0	C	566·0	B	596·0
	I	176·0	E	508·0	E	470·0	D	586·0	G	623·0
Bhasa-kalma	D	*nil*	B	353·0	G	436·0	G	523·5	D	546·0
	C	*nil*	D	452·0	D	383·0	H	517·0	I	497·0
	A	*nil*	G	499·0	I	356·5	B	552·5	F	580·5

INFLUENCE OF STORAGE ON SOME OF THE FIBRE-PROPERTIES OF DHARWAR COTTON

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(With two text-figures)

I. Introduction

In the testing of cottons for the determination of fibre-properties, it frequently happens that due to pressure of work some samples remain untested for a certain period of time. The question arises whether any change in the fibre-properties is caused by storage, if clean samples are kept for about a year under exceptionally favourable condition, as regards humidity and protection from rain, etc. The object of the present investigation is to find out whether and to what extent the more common of the fibre-properties exhibit any variation due to storage under these special conditions.

Important and extensive investigations have already been done by Ahmad [1935] on the effect of storage of *kapas* on the quality of cotton. A study of the deterioration of cotton stacked in open plinths at Karachi has also been made by the same author [Ahmad, 1934]. The work reported in this paper was undertaken in 1929 and tests on the three principal fibre-properties, length, weight and strength were carried out at an interval of six months for a period of three and a half years.

II. Experimental

(*a*) *Material*

The cotton selected for the experiments was Dharwar I as it was found by Turner [1929] to show very little variation in fibre-properties in different seasons. The test sample (season 1928-29) was kindly supplied by the Cotton Breeder, Dharwar.

(*b*) *Storage*

A sample of about 2 lbs. was preserved in a loose condition in a cloth bag, which was kept in a clean and dry cupboard. Samples were taken from this bag periodically for testing. The first sample was taken after six months and the seventh (last) in September 1932, after a period of three and a half years.

(*c*) *Sampling*

In order to get a fully representative and satisfactory sample, sampling methods described by previous writers [Calvert and Summers, 1925; Turner,

1928 ; and Clegg, 1932] were followed. Each test sample was divided into two equal parts and each sub-sample was again divided into two equal parts, and this process was repeated until there were thirty-two parts in all. From each one of these groups, small bunches of fibres were drawn and cleaned one by one. These were drawn by hand and mixed together into a sliver. The hand sliver after drawing, mixing and doubling together was passed through a draw-box several times till a representative sliver, having most of the fibres parallel and straight, was obtained.

(*d*) *Experimental procedure.*

(*i*) *Mean fibre-length.*—The mean fibre-length was determined by two instruments, (1) Balls [1916] Sorter, and (2) Baer Sorter, using the methods suggested by Ahmad [1933, 1]. The results obtained by these two instruments serve as checks upon each other, though the Balls Sorter is really the more useful instrument. Two tests were made with the Balls Sorter and four with the Baer Sorter and the mean was calculated from the results.

(*ii*) *Fibre-weight per unit length.*—In determining the fibre-weight per inch, method prescribed by Ahmad [1933, 2] was followed. Fibres were cut into one-centimetre lengths by a suitable cutter and of these about 250 fibres were counted and weighed each time on a sensitive quartz micro-balance of the type described by Morton [1926]. In all about 3,000 fibres in groups of 250 fibres per lot were weighed. From these results the mean fibre-weight per inch was calculated. As cotton is hygroscopic, the values obtained for fibre-weight depend upon the moisture content at the time of weighing and this in turn depends upon the prevailing temperature and humidity of the atmosphere. In order to have comparable values, therefore, they were all corrected to 70 per cent relative humidity using the correction factor suggested by Urquhart and Williams [1926].

(*iii*) *Fibre-strength.*—Fibre-strength was determined by the O'neill's Hair Tester as modified by Mann and Pierce [1926]. About 300 fibres were tested singly on one-centimeter lengths and from the results obtained, the mean fibre-strength was calculated.

As fibre-strength is also much affected by the changes in the atmospheric humidity, the results were corrected by using the factor recommended by Ahmad [1933, 3]. The fibre-strength per unit fibre-weight per inch was calculated from the results obtained for mean fibre-strength (ozs.) and the mean fibre-weight per inch (10^{-6} ozs.).

(*iv*) *Colour.*—During each test, the lint was examined for feel and colour. These are rather difficult tests to make, for no numerical expression can be given to them. The colour was at first creamy, but as the period of storage increased, it became more and more dull, until finally there was a marked change in its appearance.

Discussion of results

The results obtained from these experiments are given in Tables I to VI, and are graphically represented in Figs. 1 and 2.

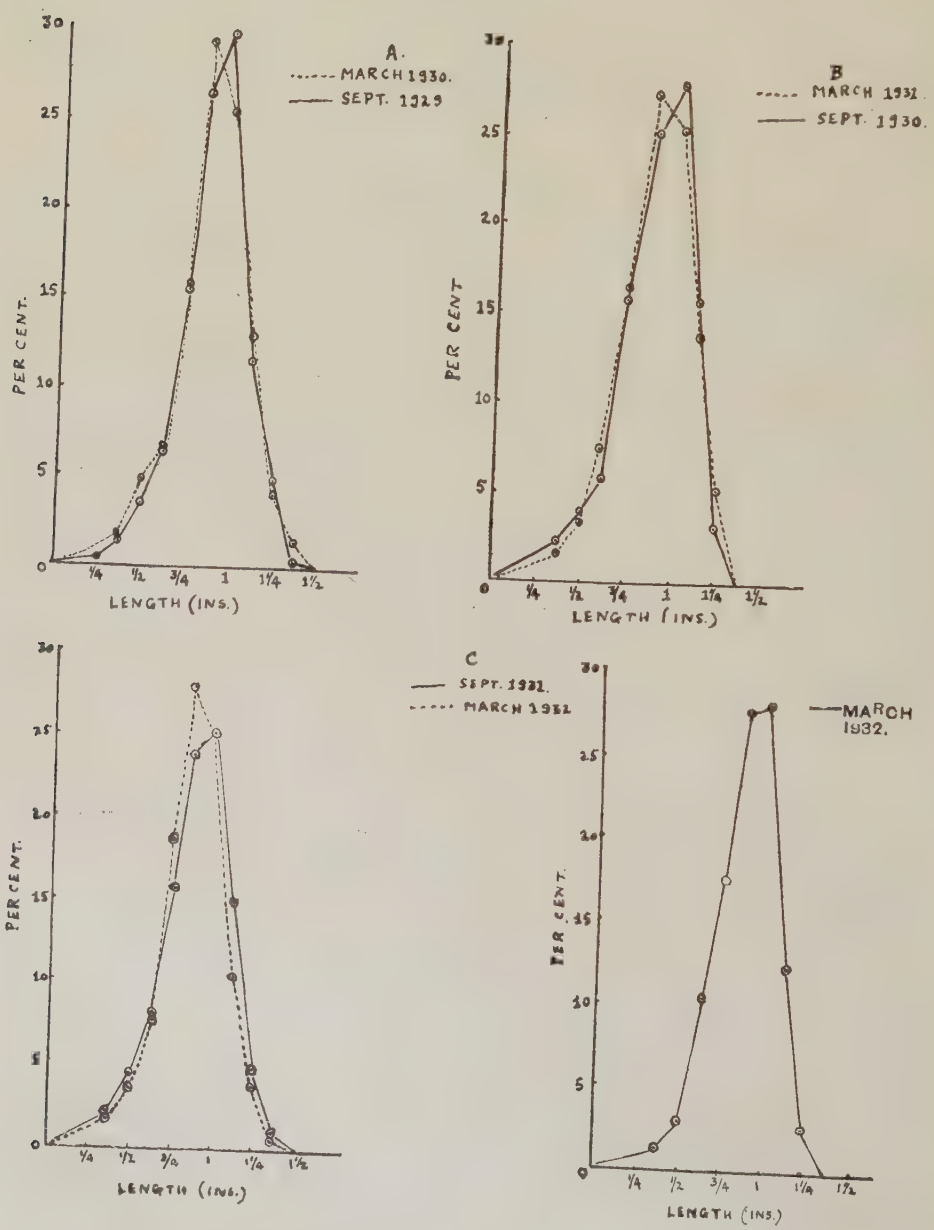

Fig. 1. Sorter diagram.

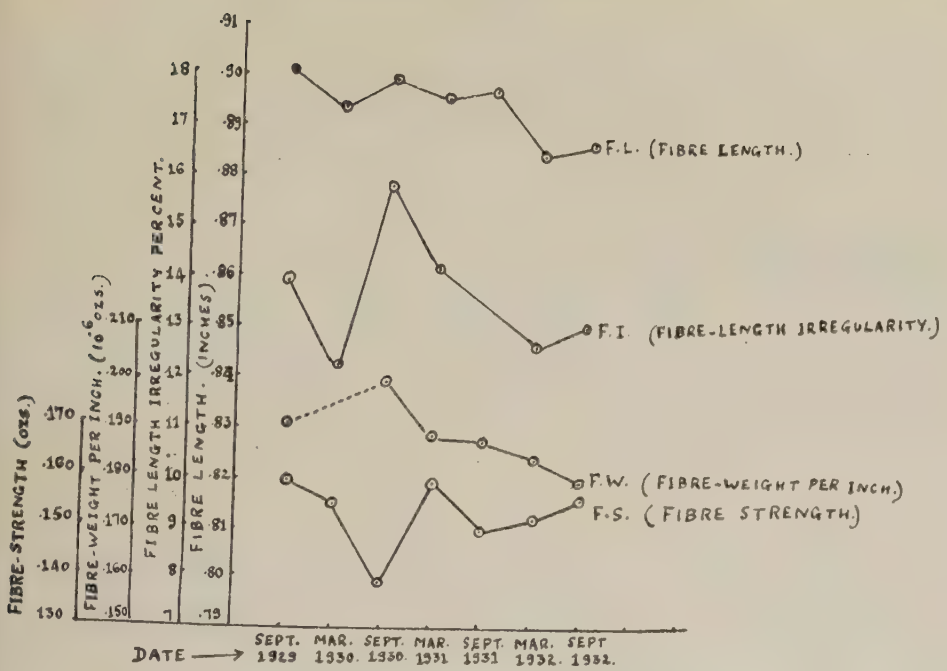

Fig. 2. Change of fibre-properties with date.

Fibre-length

Table 1 gives the mean fibre-length and fibre-length distribution.

TABLE I

Mean fibre-length and fibre-length distribution of Dharwar I (1928-29) cotton, when tested at six-monthly periods for three and a half years.

Mean group-length in eighths of an inch	Tested in						
	Sept. 1929	March 1930	Sept. 1930	March 1931	Sept. 1931	March 1932	Sept. 1932
2	0·4	..	..	..	..	..	..
3	1·3	1·6	2·0	1·7	2·0	1·7	1·3
4	3·0	4·9	3·7	3·3	4·3	3·5	2·9
5	6·2	6·4	6·3	7·7	7·9	7·8	10·2
6	15·6	15·7	16·7	15·9	15·7	18·5	17·5
7	26·6	29·2	25·1	27·1	23·7	28·0	27·7
8	29·7	25·6	27·3	25·3	25·2	25·2	28·0
9	11·8	13·1	15·7	13·8	15·1	10·5	12·4
10	5·0	4·1	3·2	5·2	4·8	3·7	3·0
11	0·4	1·4	..	..	1·3	0·8	..
Mean fibre-length (inch) (Balls Sorter).	0·910	0·905	0·900	0·900	0·904	0·887	0·895
Mean fibre-length (inch) (Baer Sorter).	0·892	0·888	0·900	0·888	0·893	0·885	0·883
Mean of Balls and Baer tests.	0·901	0·896	0·900	0·894	0·898	0·886	0·889

From the data recorded in the above table it will be noted that the Ball Sorter readings show slight variation, the highest value obtained being in September 1929 and the lowest in March 1932. The percentage difference between the highest

value and the lowest, however, is only 2 5 and this cannot be considered as significant. The Baer Sorter results show less variation, the highest being 0·900 in September 1930 and the lowest in September 1932, the percentage difference being only 1·9 which again is non-significant. It may be stated that due to errors of sampling, preparation of slivers and some inherent defects in the instruments themselves, a difference in fibre-length up to 0·04 in. may easily be obtained. These differences cannot therefore be attributed to the effect of storage. The fibre-lengths on the different dates have been graphically represented in Fig. 2 along with other fibre-properties mentioned in this paper. It will be observed that practically no variation in fibre-length occurs throughout the period of storage. In Fig. 1, the fibre-length distribution is graphically represented.

From the data of Table I, the median and mode were also calculated and the values are given in Table II.

TABLE II

Median and mode of fibre-length of Dharwar I (1928-29) cotton, when tested at different periods

Test	Mean	Median	Mode
	Inch	Inch	Inch
September 1929	0·910	0·923	0·949
March 1930	0·905	0·904	0·903
September 1930	0·900	0·919	0·959
March 1931	0·900	0·919	0·933
September 1931	0·904	0·919	0·949
March 1932	0·887	0·895	0·911
September 1932	0·895	0·908	0·934

It will be noted from Table I, that the mode is in the group-lengths 8/8ths of an inch in four out of the seven cases and the rest in 7/8 ths groups. It will be further observed from Table II that the values for mean, median and mode are slightly higher in September than in March, possibly due to the effects of humidity and temperature. The former month is characterised at Dharwar by high humidity and low temperature and this is reversed in the month of March.

Fibre-length irregularity

From the Balls-Sorter results for fibre-length distribution, the fibre-length irregularity percentage was calculated using the equation given by Ahmad [1933, 4], *viz.*,—

$$\text{Mode—Mean} = 3\,(\text{Median—Mean}),$$

$$I = \frac{100 \times S}{W},$$

where S is the weight of fibres shorter than three-fourths of the mode, W the weight of representative sample and I is the fibre-length irregularity. The results are given in Table III.

TABLE III

Values of fibre-length irregularity of Dharwar I (1928-29) cotton, obtained during different periods

	Tested in						
	Sept. 1929	March 1930	Sept. 1930	March 1931	Sept. 1931	March 1932	Sept. 1932
Fibre-length irregularity in per cent	13·9	12·3	15·9	14·3	17·1	12·7	13·2

It will be noted from the data recorded in Table III, that the values have neither increased nor decreased continuously as a result of storage and that the September values are, as before, higher than those of March.

Table IV gives the values of mean fibre-weight per inch for the various tests.

TABLE IV

Mean fibre-weight values of Dharwar I (1928-29) cotton

	Tested in						
	Sept. 1929	March 1930	Sept. 1930	March 1931	Sept. 1931	March 1932	Sept. 1932
Mean fibre-weight per inch ($\times 10^{-6}$ ozs.)	0·191	..	0·200	0·189	0·188	0·185	0·180

It will be seen from the results given above, that the fibre-weight per inch shows some variation. The fibre-weight decreases year by year, probably due to storage. The difference between the highest value obtained for September 1930 and the lowest value obtained for September 1932 is about 10·5 per cent. It has to be emphasized that the difference between successive values is not significant and some of the differences may have been also due to sampling errors. The change of fibre-weight values with date has been graphically represented in Fig. 2.

Fibre-strength

Table V gives the values of the fibre-strength and fibre-strength per unit fibre-weight per inch of Dharwar I (1928-29) cotton when tested from time to time.

TABLE V

	Tested in						
	Sept. 1929	March 1930	Sept. 1930	March 1931	Sept. 1931	March 1932	Sept. 1932
Mean fibre-strength (ozs.).	0·158	0·155	0·138	0·158	0·150	0·153	0·157
Fibre-strength / Fibre-weight	0·83	..	0·69	0·84	0·80	0·83	0·87

From the results given in Table V, it appears that there is no change in fibre-strength as the period of storage is increased ; the small difference between the highest value and the lowest is within the sampling errors. The fibre-strength per unit fibre-weight per inch is also practically constant excepting for September 1930 which is due to low fibre-strength recorded for that month.

Table VI gives the values of the frequency distribution for strength tests.

TABLE VI

Variation in the frequency distribution of fibre-strength tests of Dharwar I (1928-29) cotton, tested at different periods

Mean fibre-strength (grms.)	Frequency						
	Sept. 1929	March 1930	Sept. 1930	March 1931	Sept. 1931	March 1932	Sept. 1932
0—0·5 . .	2	3	13	5	6	7	10
0·6—1·5 . .	6	9	29	20	28	30	32
1·6—2·5 . .	21	24	37	31	46	41	24
2·6—3·5 . .	30	35	66	45	48	60	27
3·6—4·5 . .	25	28	58	51	48	72	51
4·6—5·5 . .	30	34	53	44	39	50	48
5·6—6·5 . .	17	20	30	26	38	26	40
6·6—7·5 . .	14	10	11	15	20	11	33
7·6—8·5 . .	6	2	5	10	12	6	15
8·6—9·5 . .	3	..	2	3	7	1	10
9·6—10·5 . .	1	..	..	1	1	..	2
10·6—11·5 . .	..	..	..	1	1	..	..
Mean fibre-strength (grms.)	4·40	3·99	3·70	4·20	4·19	3·72	4·55
Relative humidity per cent	66	52	60	56	63	42	75
Mean fibre-strength at 70 per cent relative humidity (grms.)	4·48	4·38	3·90	4·50	4·23	4·33	4·44
Mean fibre-strength at 70 per cent relative humidity (ozs.)	0·158	0·155	0·138	0·159	0·150	0·153	0·157
Number of fibres tested.	155	165	304	254	297	305	292

The results of the tests conducted for a period of three and a half years indicate that there is practically no change in any of the fibre-properties determined. The results apply to Dharwar I type of cotton and the present sample was stored in exceptionally favourable conditions which have very little parallelism with ordinary conditions of storage of bales in godowns and therefore the results of these conclusions cannot be held as being valid for bales stored in the ordinary fashion. As the more common fibre-properties of this cotton do not exhibit appreciable change, it is permissible to make fibre tests on stored samples of this cotton, but it has to be emphasized that these should be kept under as dry conditions as possible, for moisture induces many micro-organisms such as fungus and bacteria to attack the fibres, in which case stored cotton may suffer much damage. The effect of storage on cotton by the attack of fungus and bacteria has not been investigated in the present experiments. It may be noted, that the fact that none of the fibre-properties indicated any significant change does not necessarily imply no deterioration, for due to bacterial and fungoid infection some of the cellulose may be eaten up, thus introducing more regions of weakness, which may not be ordinarily detected by the fibre-strength test due to the weakest link already present.

Summary

The experiments described in this paper refer to the controlled conditions of storage in laboratory, where most of the harmful effects of micro-organisms and climate are eliminated. A small sample (2 lb.) of Dharwar I (1928-29) cotton was stored in a cupboard in March 1929 for a period of three and a half years, and periodic tests were made at an interval of six months commencing from September 1929. There was found to be no significant change in any of the three fibre-properties,—length, weight and strength—studied. The colour of the cotton, however, was observed to change slightly. The practical conclusion that can be drawn from these experiments is that if due to pressure of work a sample of this cotton is left untested for a year or so, it is permissable to test it afterwards for these three fibre-properties, provided due care has been taken in storing the sample.

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VARIATION IN THE FIBRE-PROPERTIES OF SIMILAR STRAINS OF COTTON GROWN IN DIFFERENT TRACTS OF GUJARAT

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(With one text-figure)

I. Introduction

The Gujarat cotton belt comprises three distinct tracts, Broach, Surat and Viramgam, each place being the nucleus of a tract. Each tract produces a typical variety of cotton. As we proceed northwards from Surat the quality falls off. An examination of the maturity of Goghari E_2 during 1931-32 showed significant difference between Broach and Surat. Similarly 1027 A. L. F. differed between Surat and Viramgam. This diversity of results for a pure type naturally gave rise to the question, whether any general rule can be found whereby it will be possible to predict how a type is likely to behave when transferred from one tract to another, under normal conditions. This question is also important in hybridisation work, since a good type evolved in one tract may be taken up for crossing in another tract. A knowledge of the probable behaviour of any variety in different tracts is thus desirable.

II. Material

With this object in view lists of all the types grown on the three farms during 1931-32 were obtained and the following types which were the only common ones, chosen for the present investigation :—(1) 1027 A. L. F., (2) Broach Deshi 8, (3) Wagad 8×1027 A. L. F.—segregates 11-2, (4) 11-4 and (5) 12-1. These represent to a great degree the three tracts—1027 the standard for Surat, B. D.-8

specially selected at Broach, and W. 8×1027 specially hybridised for the Viramgam tract For this purpose representative* samples of 4-5 ozs. of each of these types, from the general lot, were obtained from the respective cotton breeders.

III. Methods

Tests on each sample were made to ascertain the fibre-length, fibre-weight per inch, and the maturity percentage. Length is the average of two Balls-Sorter tests. Fibre weight is an average of about 2,000 whole fibres counted and weighed in bunches of 100 by the method described by Ahmad [1933, 1]. The maturity percentage is determined by a slight modification of the Clegg [1932] method; while Clegg takes fibres from different sections of a Baer-Sorter pattern according to their length, here a bunch of fibres having approximately 250 fibres is drawn from the sliver and irrigated with eighteen per cent caustic soda solution after placing them between a micro-slide and a cover glass. A minimum of 500 fibres are tested for each sample. The rest of the experiment is similar to Clegg's.

IV. Discussion of results

1. Fibre-length

Table I below gives the average values of fibre-length in inches for all the samples tested. The percentage differences in length between the several tracts are also given.

Table I

Mean length and percentage differences in mean length in inches

No.	Cotton	Surat S	Broach B	Viramgam V	S—B	$\frac{S-B}{S}\times\frac{100}{1}$	V—B	$\frac{V-B}{V}\times\frac{100}{1}$	V—S	$\frac{V-S}{V}\times\frac{100}{1}$
1	1027 A. L. F. .	0·95	0·93	0·94	0·02	2·1	0·01	1·0	—0·01	—1·1
2	B. D.-8 . . .	0·93	0·89	0·87	0·04	4·3	—0·02	—2·3	—0·06	—6·9
3	W.-8×1027 11-4 .	0·87	0·94	0·87	—0·07	—8·1	—0·07	—8·0	0·00	0·0
4	W.-8×1027 11-2 .	0·90	0·89	0·90	0·01	1·1	0·01	1·1	0·00	0·0
5	W.-8×1027 12-1 .	0·89	0·90	0·89	—0·01	—1·1	—0·01	—1·1	0·00	0·0

* A representative sample was obtained thus:—The available lint was divided into eight equal parts. Portions from every part were taken in turn and mixed with every other part; the process being repeated a dozen times so that lint from each of the original parts was well distributed in a random manner in every other part. From all these thoroughly mixed parts small tufts were taken from different sides of each part and mixed together to form this sample.

We find therefrom that between Surat and Broach the mean length varies from 1·1 to 4·3 per cent for four cottons and 8·1 per cent for one cotton. Between Surat and Viramgam the variation is *nil* in three cases and 1·1 and 6·9 in two cases. Between Broach and Viramgam the variation is only 1·1 to 2·3 per cent for four cases and 8 per cent in one case. Further the differences are sometimes positive and sometimes negative and are also within experimental errors in most of the cases. Three exceptions where the observed differences are outside the experimental errors may be noted, *viz.*, B. D.-8 at Surat has greater mean length than at Viramgam and even than at Broach, where it was selected; and W.-8×1027, 11-4, which has an average length of 0·87 in. both at Surat and Viramgam shows a length of 0·94 in. in Broach.

We therefore conclude that there is no definite trend of variation in mean length (either towards lengthening or shortening) from one tract to another in general, though some variation may be expected in particular varieties like B. D.-8 which becomes shorter as we proceed from Surat through Broach to Viramgam.

2. Distribution of fibre length.

In Table II the frequency distribution of fibres according to their length is given.

TABLE II

Showing frequency distribution of fibre-length

1/8-in.	1027			B. D.-8			11-4			11-2			12-1			Remarks
	S	B	V	S	B	V	S	B	V	S	B	V	S	B	V	
2	0·8	..	0·5	..	..	0·5	0·9	..	0·7	..	..	0·7	..	..	0·5	
3	2·0	1·3	1·3	1·2	1·0	1·6	2·0	1·2	2·1	1·9	1·0	1·5	2·7	1·2	1·4	
4	2·9	2·1	2·0	1·5	2·0	2·1	3·2	1·9	3·1	3·1	2·0	2·2	3·7	1·5	1·8	
5	4·5	4·2	4·4	3·4	4·8	9·3	6·0	4·4	8·2	6·9	5·0	5·2	6·5	5·5	6·4	
6	9·3	12·2	11·4	10·7	15·8	16·3	16·5	11·6	19·7	15·0	17·2	15·9	15·0	16·0	18·5	
7	20·4	27·7	23·4	32·9	38·9	35·3	34·7	26·3	33·4	27·6	38·5	30·4	29·4	34·8	31·0	
8	31·2	36·1	32·8	36·2	29·8	24·2	26·8	34·6	22·1	28·3	24·3	27·5	26·8	25·4	26·5	
9	20·0	11·2	17·7	9·4	6·0	9·2	7·6	13·9	7·5	12·8	9·0	12·0	12·0	10·6	9·5	
10	7·4	3·7	4·7	3·3	1·2	1·5	2·3	4·1	3·2	3·4	2·0	3·3	2·8	3·5	2·8	
11	1·5	1·5	1·8	1·4	0·5	..	..	2·0	..	1·0	1·0	1·3	1·1	1·5	1·6	

S=Surat, B=Broach, V=Viramgam.

We notice in Table 11 that if we take the modal group for any type in the three tracts, the Broach sample has the maximum percentage of fibres. For instance for 1027, 8 is the modal group for all the tracts ; and in this Broach has a maximum of 36·1 per cent, Surat 31·2 per cent and Viramgam 32·8 per cent of fibres. Except for 11-4, where there is practically no difference among the tracts, Surat and Viramgam are similar while Broach has a greater percentage. A maximum difference of 10·9 is found in the modal value between Broach and Surat for 11-2.

The frequency curves for these distributions are given in Fig. 1. All the curves are practically of the same type and there is considerable overlapping of the curves for any sample from the three tracts. This is particularly noticeable in the case of B. D.-8, 1027, 12-1 and 11-4. In 11-2 the Broach curve stands prominent with a sharp peak. Even though the variations in mean length are not significant these curves show that the percentage distribution of fibres of different lengths is slightly altered from tract to tract. This is well brought out when the figures are compared from group to group.

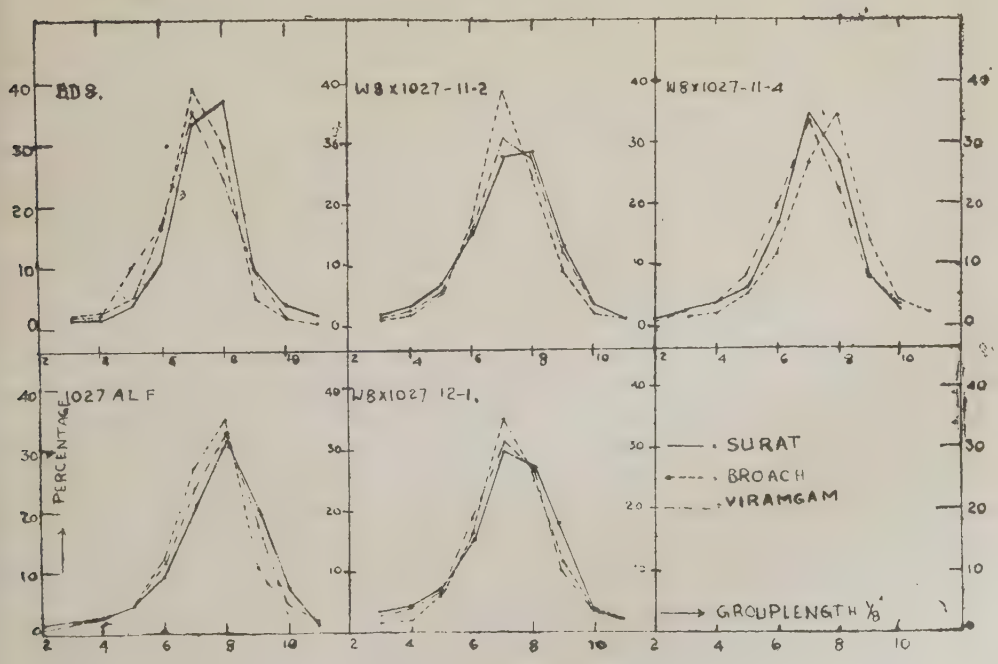

Fig. 1. Fibre-length tribution.

3. Irregularity percentage

From the frequency-distribution table we can calculate the irregularity percentage which, as is shown by Ahmad [1933, 2] is a statistical measure giving the percentage of fibres less than three-fourths of the modal length. Obviously the less the percentage the greater the spinning quality, all else remaining same. Table III below gives the percentages for all the samples.

TABLE III

Irregularity percentage and mean differences

No.	Sample	Surat S	Broach B	Viram-gam V	S—B	V—B	V—S
1	2	3	4	5	6	7	8
1	1027 A. L. F. . .	15·0	12·1	14·8	2·9	2·7	—0·2
2	B. D.-8 . . .	10·7	7·0	10·5	3·1	2·9	—0·2
3	W.-8 × 1027 11-4 .	13·3	12·0	13·8	1·3	1·8	0·5
4	W.-8 × 1027 11-2 .	15·1	7·5	12·0	7·6	4·5	—3·1
5	W.-8 × 1027 12-1 .	15·5	8·3	13·0	7·2	4·7	—2·5

Between Surat and Viramgam the percentage in any one type differs from —3·1 to plus 0·5, a negligible difference, between Surat and Broach it varies between 1·3 and 7·6 per cent and between Viramgam and Broach 1·8 to 4·7 per cent. In the latter two cases even though the differences are not very great for all types, it is uni-directional and in two cases more than 7 per cent. An error of two per cent may in general be assumed to occur in the determination of this property and thus the difference between Viramgam and Surat becomes insignificant except for 11-2 and 12-1. From columns 6 and 7 of the above table we also find that with the exception of 11-4, all the differences between Broach and either Surat or Viramgam are outside the experimental error of two per cent, the Broach samples always having a lower irregularity percentage. Besides, for 11-2 and 12-1 the differences between Surat and Broach are greater than thrice this error and are even statistically significant.

From these facts we may infer that the samples grown in Broach are more regular in staple than those grown either at Viramgam or Surat, except for 11-4 which shows a very small difference ; while there is no difference in this respect between Surat and Viramgam except for 11-2 and 12-1.

4. Fibre-weight per inch

This in general is a quantitative measure of the fineness of a sample. Table IV below gives the results of fibre-weight per inch for all the samples in 10^{-7} ozs.

TABLE IV

Fibre-weight per inch and percentage mean differences

No.	Sample	Surat S	Broach B	Viram-gam V	S—B	$\frac{S-B}{S}\times\frac{100}{1}$	V—B	$\frac{V-B}{V}\times\frac{100}{1}$	V—S	$\frac{V-S}{V}\times\frac{100}{1}$
1	1027 A. L. F. .	1·58	1·57	2·07	0·01	0·6	0·50	24·2	0·49	23·7
2	B. D.-8 . . .	1·71	1·56	1·92	0·15	8·8	0·36	18·8	0·21	10·9
3	W.-8×1027 11-4 .	1·84	1·52	1·97	0·32	17·4	0·45	22·8	0·13	6·6
4	W.-8×1027 11-2 .	1·86	1·64	2·00	0·22	11·8	0·36	18·0	0·14	7·0
5	W.-8×1027 12-1 .	1·49	1·43	1·80	0·06	4·0	0·37	20·6	0·31	17·2

The fibre-weight for any one sample varies from 0·01 to 0·32 units between Surat and Broach, from 0·36 to 0·50 between Viramgam and Broach and from 0·13 to 0·49 between Surat and Viramgam. Expressed as percentages, Broach samples are finer than either Surat or Viramgam by 0·6 to 17·4 per cent and 18·0 to 24·2 per cent, respectively. The Surat samples are finer than Viramgam ones by 6·6 to 23·7 per cent. Since the maximum error in the fibre-weight determination would be about six per cent, most of these differences are statistically significant It therefore appears that in general these types have a graded variation from tract to tract ; though the amount of this variation is different for different types, each reacting differentially to the internal and external environmental factors of soil and atmosphere respectively, depending upon their inherent capacities. The effect of tract is most pronounced for coarseness in case of 1027 as between Viramgam, and Broach or Surat, while it is practically *nil* as between Surat and Broach. Between Broach and Viramgam the differences are particularly high and roughly uniform for all cottons, varying in percentage from 18·0 to 24·2. 11-2 and 11-4 have a markedly higher fibre weight in Surat than in Broach.

Judging the results as a whole we conclude that the types are coarsest in Viramgam and finest in Broach, Surat being assigned an intermediate position.

5. Fibre-maturity

As has been pointed out earlier the genesis of the present investigation lies in the observed differences in this property. Table V below gives the maturity results for all the samples. The first number gives the percentage of mature hairs and the second one percentage of dead hairs. The difference between the sum of these two and 100 giving the percentage of half-ripe fibres.

TABLE V

Fibre-maturity percentage and mean differences

No.	Sample	Surat S	Broach B	Viram-gam V	S—B	V—B	V—S
1	1027 A. L. F. . .	75—8	56—9	80—6	19—$\bar{1}$	24—$\bar{3}$	5—$\bar{2}$
2	B. D.-8 . . .	86—3	70—6	90—1	16—$\bar{3}$	20—$\bar{5}$	4—$\bar{2}$
3	W.-8 × 1027 11-4 .	58—7	57—11	76—4	1—$\bar{4}$	19—$\bar{7}$	18—$\bar{3}$
4	W.-8 × 1027 11-2 .	76—5	63—8	79—4	13—$\bar{3}$	16—$\bar{4}$	3—$\bar{1}$
5	W.-8 × 1027 12-1 .	65—9	61—6	73—5	4—3	12—$\bar{1}$	8—$\bar{4}$

$\bar{1}$ = minus 1.

Considering first the percentage of dead hairs we note that the differences between any two tracts or samples themselves are very small and we may hence conclude that there is no variation in this respect. Considering the percentage of mature hairs, we find that in every type there is a gradation from tract to tract. Compared with Surat, Broach has a lower percentage varying from one to nineteen and Viramgam a higher percentage ranging from three to eighteen; and Viramgam has twelve to twenty-four per cent more mature hairs than Broach. In this test a difference of eight per cent and more in any one class of hairs is considered significant. Applying this criterion we find that as between Viramgam and Surat the maturity difference is significant for only 11-4. It is significant for three samples 1027, B. D.-8 and 11-2 as between Broach and Surat, while it is significant for all cottons as between Broach and Viramgam. Thus the difference in the degree of hair-maturity varies from tract to tract as between cotton and cotton.

These differences, which are significant, lead us to conclude that the Broach samples are the least mature, Viramgam samples containing the highest percentage of mature hairs, while the Surat samples occupy intermediate positions. The differences which are not significant are however also in the same direction as the significant ones. This variation which is uni-directional goes hand in hand with the fibre-weight results noted earlier. The tract with a maximum fibre-weight has a maximum maturity and the finest variety tract the lowest maturity, and the third one intermediate in both cases. It must be noted however that these are relative differences among these three tracts only.

One ore point emerges from the correlation of these two results. The facts that the finest (Broach) is least mature and, conversely, the coarsest (Viramgam) most mature taken in conjunction with each other, indicate that **relatively** the fibres will be found weakest in Broach and strongest in Viramgam.

These results obtained for three different types indicate that we may possibly expect similar results to hold good for another type when transferred from one tract to another provided the season is a normal one, because tract includes (*i*) soil, and (*ii*) external environment. Soil is generally same in a tract and thus the changes are truly reflected provided there is no abnormality in the external conditions confined to only one tract. Given a normal season, when no particular tract was singled out for abnormal atmospheric conditions, the five samples tested gave results leading to the following conclusions.

V. Conclusions

There is no significant variation in mean fibre-length of most of the types discussed above, when grown on the different farms of Broach, Surat and Viramgam. However, B. D.-8 tends towards decreasing mean length as we proceed from Surat through Broach to Viramgam, and strain 11-4 tends to be longer in Broach than either at Surat or Viramgam.

The irregularity percentage is least for Broach and similar as between Surat and Viramgam except for 11-2 and 12-1.

The fibre-weight per inch and hence the coarseness of samples is least in Broach, highest in Viramgam and intermediate in Surat, all the observed differences being beyond experimental errors except for 1027 and strain 12–1 as between Surat and Broach which however are in the same direction as the other differences.

Similarly the maturity percentage is maximum in Viramgam and minimum in Broach, Surat being intermediate. The differences are significant for all cottons as between Viramgam and Broach, for three as between Broach and Surat and for one as between Viramgam and Surat. The other differences are in the same direction as the significant ones.

The high maturity and high fibre-weight per inch in Viramgam and the low maturity and low fibre-weight per inch in Broach taken in conjunction with each other respectively indicate a probable **relative weakness** in Broach samples as compared with similar ones of Viramgam.

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DISEASES OF *CITRUS* IN THE PUNJAB

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(With Plates XII-XIX and one text-figure)

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I. Introduction

Citrus plants in India suffer from a number of serious diseases, causing considerable losses, and very little has so far been done to study them with a view to controlling them. Lately, however, in several parts of India, the study of *Citrus* diseases has been undertaken. The important *Citrus*-growing provinces in India are Central Provinces, Assam, Punjab and Bombay. In Central Provinces, Bombay Presidency and in Assam mostly the loose-skinned mandarin group of oranges are grown, whereas in the Punjab, the close-skinned *malta* oranges form the most important *Citrus* fruits. Recently great strides have been made in *Citrus* growing in India. This has been mainly due, thanks to the Imperial Council of Agricultural Research, India, to better marketing facilities and horticultural experiments for introducing better varieties. The climatic conditions in all these provinces differ very much and so do the fruits. Lime (*Citrus aurantifolia*) is grown practically all over India and canker is almost universally associated with it, and is one of the most prevalent *Citrus* diseases. True scab, however, is very rare, but it has been definitely noticed in the Punjab and in Saharanpur Gardens and also in the South in the Madras Presidency. Anthracnose (wither-tip) due to *C. gloeosporioides* is found practically all over India but only in the Punjab it has become one of the most serious diseases of the *Citrus* plants. Die-back of orange plants in the Bombay Presidency associated with *Diplodia* sp. has been more often referred to as due to bad aeration, and is claimed to have been controlled [Sahasrabuddhe, 1927 ; Cheema and Bhatt, 1929] by proper aeration of the soil. A gummosis due to *Phytophthora palmivora* Butl. and a root-rot due to *Rosellinia* sp. have also been reported from Bombay Presidency besides anthracnose (*C. gloeosporioides*). Similarly from South India anthracnose and pink disease (*Corticium salmonicolor* B. et Br.) have been reported. The sooty mould is very common in many parts and generally follows in the wake of scale-insects. It may lower the vigour of the tree by completely and heavily encrusting all the leaves of the trees. In very bad cases, fruit-fall may be caused.

In the Punjab, almost all the varieties of *Citrus* fruits are to be found, but it is only the loose-skinned *santara* and the close-skinned *malta* oranges that are cultivated on a commercial scale. The lime (*nimbu*), citron (*galgal*), pomelo (*chakotra*), etc., are also being cultivated more and more. The sour orange (*khatta*), and the rough lemon (*khatti*) are extensively grown as hedge plants

throughout the Punjab. The rest are not grown on a commercial scale but are often found scattered individually in private gardens.

Throughout this country there is great confusion about the identity and classification of the *Citrus* varieties. Different botanists have formulated different classifications, establishing the species and varieties on not very reliable and stable characters with the result that the calssification of *Citrus* plants is in a state of hopeless confusion. This confusion has become worse owing to the readiness with which several species hybridise and also perhaps, as Bonavia [1888] says, owing to changes brought about in a new environment when plants are first introduced. Brandis [1874], Bonavia [1888], Lushington [1910], Swingle [1914], Coit [1915], Fawcett and Rendle [1920], Davis [1924], Parker [1924], Hume [1926], Tanaka [1927, 1930] and others have given different classifications and descriptions with the result that the selection of the correct botanical name is extremely difficult. Recently Cheema and Bhatt [1934] have studied the *Citrus* varieties of the Bombay Presidency and have recommended Swingle's classification [1914] for adoption. The confusion that at present prevails may be seen from the following different botanical names that have been applied by different botanists to the loose-skinned *santara* orange, *viz.*, *Citrus nobilis* Lour ; *C. nobilis* (Lour.) var. *deliciosa* Swing. ; *C. deliciosa* Tenore ; *C. poonensis* Tanaka ; and *C. aurantium sinense* Rumph.

In this paper the vernacular names as used in the Punjab, have been used throughout, for the work was undertaken with a view to help the *Citrus* growers of the Punjab and the references to the plants must be easily understood by them.

Advantage was taken of Professor Tanaka's recent visit to Lahore. The author had all the local varieties examined and named by him and the following list shows the vernacular names, with their botanical and nearest English equivalents, referred to in this paper. He is greatly indebted to Prof. Tanaka for this.

Vernacular name	Botanical name	Nearest English equivalent
Malta	*Citrus sinensis*	Sweet orange
	NOTE.—Introduced from Europe into Gujranwala by Col. Clark, 1852-56. The Gujranwala blood-red oranges are still famous throughout the province.	
Santara	*Citrus poonensis*	Mandarin type
Mitha	*Citrus limetta*	Sweet lime
Khatti (*Gambhiri*)	*Citrus jambhiri*	Rough lemon
Khatta	*Citrus aurantium*	Sour orange
Chakotra	*Citrus grandis*	Shaddock or Pomelo
Grape fruit	*Citrus paradisi*	Grape fruit
Galgal (big)	*Citrus medica*	Coarse citron
Galgal (small)	*Citrus limon* var.	Lemon type
Nimbu (lemon)	*Citrus limon*	Lemon
Nimbu (*Kagzi*)	*Citrus aurantifolia*	Lime

The following diseases of the *Citrus* plants occurring in the Punjab, *viz.*, the 'wither-tip,' (*Colletotrichum gloeosporioides* Penz.), sooty mould and chlorosis are dealt with in this paper. The anthracnose (wither-tip) causes the greatest loss to the *Citrus* in the Punjab, and this disease has now been studied in detail and remedial measures have been suggested.

II. Wither-tip disease.

(A) Anthracnose (wither-tip) caused by *Colletotrichum gloeosporioides* Penz.

(*a*) *Review*

This disease is found throughout the province and can be seen on most of the *Citrus* species, though it is more common on *santara* and *malta* (Plate XVI, figs. 1, 2). The diseased tips become dead and assume a silvery grey appearance on which minute dark acervuli of *Colletotrichum* soon appear. The healthy portion becomes sharply demarcated from the diseased portion (Plates XV). The disease, however, is not equally severe in all parts of the province and has been noticed in the following places, *viz.*, Peshawar, Wah, Rawalpindi, Jammu, Udhampur, Pathankot, Nurpur, Kangra, Baijnath, Jullundur, Hoshiarpur, Saharanpur, Delhi, Patiala, Lahore, Amritsar, Gujranwala, Daska, Ranbirsinghpura, Udera, Mangtanwala, Sheikhupura, Sarakpura, Bahawalpur, Renala, Multan and Dehra Gazi Khan (Fig. 1.) It has been noticed that generally along the low hills and places within a hundred miles from the foot of the hills the most badly affected orchards are found. But it has been felt all the same that the prevalence of the disease in an epidemic form is due more to ignorance and neglect than to anything else. For instance, in the Gujranwala-Daska area which is a big *Citrus*-growing centre with a large number of old and new plantations, the wither-tip disease prevails in a rather severe form throughout. In many of the old gardens dying or dead plants are left in the field which act as a perpetual source of infection to the other healthy plants. In the newer orchards, two or three years old, most of the plants were found to be already affected. This was primarily due to the fact that the growers not having any idea about the disease started with diseased stocks, and, in two or three years' time when they would expect the yield, perhaps many of the orchards would have to be cut down altogether after about five years of ignorant care and attention and a loss of good deal of money and energy. This will no doubt be a great set-back to the growing industry. It is difficult to find nurseries in the Punjab free from wither-tip and canker diseases. Chaudhuri and Singh [1930] found the anthracnose organism (*C. gloeosporioides* Penz.) in many parts of India, but nowhere it occurred in such epidemic form as in the Punjab. Though it has often been looked upon as causing a secondary infection, there is no doubt that in many parts of the

world it occurs as a serious parasite of *Citrus* plants and causes great loss. Rolfs [1904], Essig [1911], Barrett [1915] and others, and in recent years Burger [1923], Peyronel [1926], Reichert [1928], Muller [1933] and others have found it parasitic on different *Citrus* species. Petri [1926] proved its parasitic nature on lemon by means of inoculation experiments. He also [1927] considered it to be the cause of *mal secco*—a very serious disease of *Citrus* in Italy. Later however [1928, 1929], he doubted its being the cuase and found [1926; 1930, 1; 1930, 2; 1931] *mal secco* to be due to a new fungus which he named *Deuterophoma tracheiphila*. The two fungi are very often associated, and Petri [1930, 2] has compared the characteristics of the two. Savastano and Fawcett [1930] and others have recently confirmed Petri's diagnosis regarding *mal secco*.

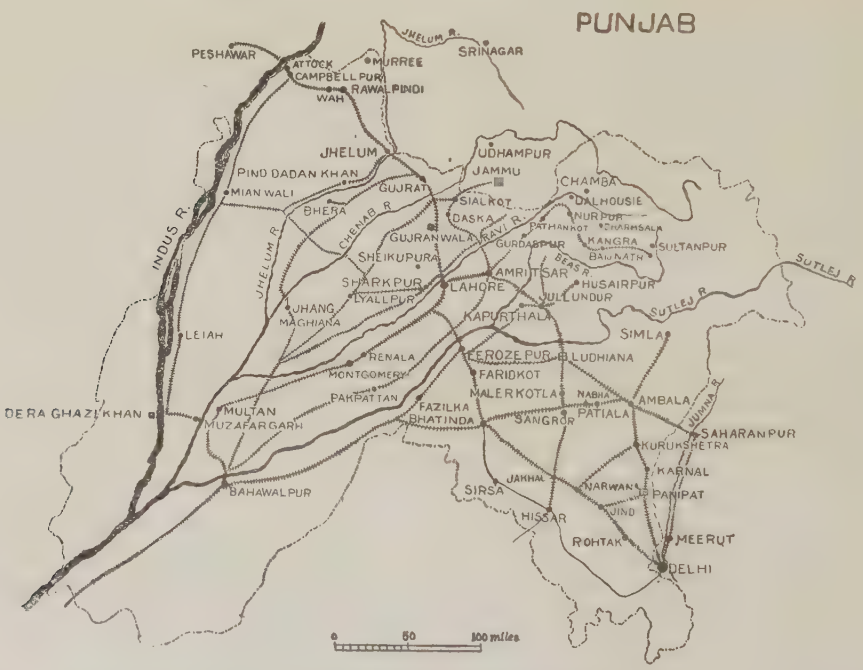

Fig. I. Map of the Punjab showing places where wither-tip of the *Citrus* has been noticed.

Another similar anthracnose-producing organism, *Gloeosporium limetticolum* Claus. frequently causes serious damage to limes in Florida, Cuba and West Indian Islands, and it has been found by Fulton [1925] and others that in those countries *C. gloeosporioides* Penz. only attacks later on when the plants have been weakened by the attack of *Gloeosporium*. A serious wither-tip disease of limes from Dominica, due to *G. limetticolum* and causing very similar symptoms to *C. gloeosporioides* wither-tip, has been described by Steli [1924], Wiltshire [1925] and Ashby [1925]. It threatens to destroy the crop in some parts of the

island. The lime wither-tip was first described by Watts [1923] as being due to *C. gloeosporioides*. In fact the two organisms are very much alike. In *C. gloeosporioides* Penz. the pustules are frequently devoid of setae and in that condition it is difficult to distinguish it from *G. limetticolum* Claus. Petri [1927] also found that the setae are not regularly produced in the acervuli and often are completely absent. *C. gloeosporioides* is very variable [Burger, 1921] and several strains which differ in their virulence have been found. Chaudhuri and Singh [1930] and Muller [1933] isolated distinct forms from different *Citrus* species.

Though the fungus is quite commonly found on dead twigs of *Citrus*, which may have been killed by other organisms or by any other cause, there is no doubt, however, about its power of causing disease unaided, as has been proved by a large number of inoculation experiments. It has been very frequently observed, as mentioned by Fawcett [1915], that some of the severest injuries in connection with this disease may be due to the combined effect of anthracnose fungus and other fungi, such as *Diplodia*, *Phomopsis*, etc. Also trees previously weakened by unfavourable soil conditions, such as continued low temperature, excessive frosts, etc., become more susceptible to this disease. In 1929 great loss occurred when, for three successive days during January, Lahore experienced a cold wave and had 15 to 20° of frosts everyday. The *Citrus* plants were chilled and weakened and shortly afterwards most of the plants in the nurseries near-about were attacked and killed by the wither-tip organisms. It has also been noticed that injuries produced by a severe hail-storm made the entry of the wither-tip organisms easy. In Plate XIX, fig. 1 a twig which had been injured by hail-storm and later became infected with *C. gloeosporioides* Penz. is depicted. Barrett [1922] reported anthracnose on fruits when injured by hail-stones.

The wither-tip of *Citrus*, though a minor disease in other parts of India, has become a major one in the Punjab. There is nothing to be wondered at in this. For it is a well-known fact that " the differences in climate and other conditions between two countries or even two localities in the same country, may be so great as to make diseases that are major in one place, minor or of no economic importance in the other. Major diseases of one variety may be minor in another " [Fawcett and Lee, 1926]. Even in the Punjab it has been noticed that the virulence of the disease differs in different parts of the province, which however may be due to having several strains of *C. gloeosporioides*, some being more vigorous than the others.

(*b*) *Symptoms*

In Plate XV twigs of *Citrus* affected by wither-tip due to *Colletotrichum gloeosporioides* are shown. The sharp demarcation of the diseased portion from the healthy part and their silvery-grey appearance are very characteristic. The acervuli of the fungus may be seen as black dots scattered throughout the dead part of the twig (Plate XV). In moist weather these may be seen bursting when

pinkish masses of spores are seen oozing out. The setae may be seen frequently around these bodies. These may be either continuous or septate. In the anthracnose spots of the leaves, the acervuli are found on the upper surface and are half submerged in the tissue of the leaf (Plate XIX, fig. 2).

(c) *Morphology*

Acervuli and spores.—The diameter of the acervuli ranges from 60 to 270μ. The size of the spores varies a good deal and even in the same acervulus a lot of variation in spore sizes is seen. On measuring one hundred spores at random the length of the spores has been found to vary between 11·2 and 21·0μ and the width between 2·4 and 7·0μ. The mean values of length and breadth of spores from a malta twig were found to be 13·2 and 5·5 μ, respectively.

Spores are somewhat greenish when young and are full of protoplasmic granules and oil globules. Later they become somewhat hyaline. When mature, spores ooze out in mass from the acervuli; they appear pinkish in colour. They have usually one nucleus but frequently two nuclei have also been noted. Spores germinate readily. In hanging drops germination was observed in four hours. Germ-tubes are usually formed from one end though sometimes they may be formed from both the ends and the hyphae produced may be seen forming conidia in four to five days' time (Plate XII, *c*, *d*, *e*). The apex of the conidiophore becomes swollen, into which protoplasmic granules, etc., pass and become cut off from the rest of the conidiophore, thus forming a conidium. When it is abstricted, another conidium will be formed in the same way. Sometimes when a number of spores are put in a hanging drop, it is common to find the spores become septate at the time of germination by the formation of a partition wall.

(d) *Growth in culture*

Single spore cultures were taken in this study. It grows well on several media such as potato glucose and potato extract media, pea, bean, prune, gram, wheat and barley agar and also on Coons', Claussen's, Barnes', Raulin's, Czapek's and Richards' media. In the preparation of the first series of media, excepting in potato glucose, five grms. of materials were taken for every 100 c.c. of water and two per cent of agar was added. For prune agar, more agar was added if necessary. Potato glucose agar and the different synthetic media were prepared in the usual way. Petri dishes of uniform size (90 mm.) were used and 20 c.c. of the media poured to have a uniform thickness. In Table I, the growth characters and the total spread of the fungus isolated from a diseased *malta* twig have been shown in some of the above media.

TABLE I

Growth character and spread of C. gloeosporioides *in different media at room temperature*

Media	Growth character at room temperature (about 26°)	Spread in six days (diameter in mm.)
Pea agar . . .	Cottony hyphae, not very luxuriant, acervuli produced in ring which soon burst oozing out pink mass of spores. No blackening of reverse surface.	54
Bean agar . .	Woolly hyphae, aerial hyphae erect. Pinkish spore masses appear early only near the centre, reverse side not blackened.	45
Potato glucose agar .	White fluffy growth, reverse darkened, acervuli appear late, formed near centre.	45
Prune agar . .	Profuse aerial growth, white at first, later somewhat greyish and finally almost black. Many big-sized acervuli. Reverse blackened.	60
Barley agar . .	Hyphae poorly developed, moderate, acervuli with setae, usually in rings. Reverse side becomes blackned later.	35
Maize agar . .	Aerial growth poor, acervuli few . . .	47
Czapek's agar . .	Dense woolly growth, limited number of acervuli, reverse side turns black.	45
Coons' agar . .	Scanty mycelial growth, reverse almost transparent, limited minute acervuli in a ring.	45
Richards' agar . .	Aerial hyphae almost absent, many acervuli .	62
Dox' agar . .	Dark radiating hyphae, reverse almost black, a few acervuli in centre.	55

In Plate XVII, figs. 1-4, the growth characters in potato glucose, prune, Coons' and Dox' media are shown.

The length and breadth of the spores on different media varied a good deal and the smallest sized spores were found when cultures were grown in plain agar. The mean values for length and breadth, using 100 spores, are given in Table II.

TABLE II

Mean values of length and breadth in μ of C. gloeosporioides *spores in certain media*

Media	Length	Breadth
Plain agar	9·6	3·8
Barley agar	12·3	4·2
Pea agar	13·5	4·5
Potato agar	15·2	5·0
Raulin's agar	15·1	5·0
Richards' agar	16·0	4·0
Barnes' agar	18·8	4·9

Since the growth character and the size of the spores were found to depend on the media employed, it was decided to grow the fungus on different concentrations of media and to study the effect of concentration on growth. For this two synthetic media, Richards' and Czapek's were selected. Petri dishes were inoculated with a spore suspension placed in the centre and these were incubated at 25°C.

It has been found that the greatest spread of the mycelium takes place at normal concentrations of the two media and that it decreases regularly either way when the concentrations are increased or decreased. Regarding the length

of the spores, it has been found that in both the media it increases from the lower to the higher concentrations. No such regularity was observed regarding width. Though the length of the spores increased in higher concentrations, the number of spores found in very high concentrations was fewer and at 4*N* in Czapek's no spores were found in nine days' time.

(*e*) *Strains of* C. gloeosporioides

Chaudhuri and Singh [1930] mentioned the possible existence of strains, and referred to three probable strains isolated from different *Citrus* species which showed difference in spore measurements. Though a large number of cross-inoculations were done, each strain succeeded in infecting all the other hosts. Beside slight morphological differences, no physiological differentiation was found. This work has now further been extended and four different strains from different hosts have been isolated which show decided difference in their virulence and growth characters. There are possibly many other strains. The detailed descriptions of the four strains isolated have been given later. The difference in the size of the spores is not a fundamental criterion in determining a strain without considering various other characters. As has already been shown the spore-size not only varies with difference in the kind of nutrient media on which it is grown, but also depends on temperature, and it has been found in nature that even on the same host, the size of the spore varies in different seasons. The average spore sizes (counts of fifty) in microns from different varieties in two seasons are given below :—

	September.	May.
Musumbi	13·7 × 4·1	14·4 × 3·9
Excellence	14·5 × 4·1	14·9 × 3·7
Italian	14·0 × 4·2	14·0 × 4·5
Blood Red	13·6 × 4·0	14·6 × 3·9
Jaffa	14·5 × 4·2	12·9 × 3·8

The average measurements of spores (counts of fifty) on potato glucose agar at room temperature from different plants in the first generation of culture were taken and those forms showing difference in spore-measurements were first grown under different physiological conditions for selecting the strains. From these only those showing permanent differences were chosen.

In Table III, the total amount of spread of hyphae in three different media and variation in spore measurements of certain of the above forms isolated from *malta*, *santara*, *khatta*, lemon, *galgal* and *mitha* have been shown. Culture from each has been grown at two different temperatures, *viz.*, 25°C. and 30°C. and the difference in the total spread and spore size at these two temperatures will be seen.

TABLE III

Effect of different media and temperatures on the spread and spore size of C. gloeosporioides *isolated from different host plants*

Host	Temperature (° C.)	Maize extract agar		Potato glucose agar		Czapek's agar	
		Spread in mm.	Spore size (average o 50) in μ	Spread in mm.	Spore size (average of 50) in μ	Spread in mm.	Spore size (average of 50) in μ
Malta	25°	60	14·3 × 5·2	55	12·7 × 4·1	70	13·2 × 4·2
	30°	70	13·5 × 5·3	70	12·1 × 4·1	70	14·2 × 4·2
Santara	25°	60	11·6 × 4·0	67	11·8 × 4·0	70	15·5 × 3·9
	30°	72	12·5 × 4·6	80	14·6 × 3·7	72	14·2 × 4·7
Khatta	25°	63	14·8 × 5·0	66	13·4 × 4·8	73	12·3 × 3·8
	30°	80	13·2 × 4·2	80	14·5 × 4·4	74	11·7 × 3·9
Lemon	25°	60	13·3 × 4·6	64	...	70	12·5 × 3·2
	30°	70	11·6 × 3·8	70	...	70	12·2 × 3·7
Galgal	25°	59	12·5 × 4·6	64	11·2 × 4·1	70	12·3 × 3·4
	30°	75	12·3 × 5·2	80	...	76	13·8 × 4·2
Mitha	25°	62	11·2 × 3·8	70	11·4 × 3·9	70	...
	30°	70	10·2 × 4·7	77	14·0 × 4·7	77	

From the large number of parallel cutures grown and from the study of their behaviour under different physiological conditions strains A to L were isolated, and of these the following four were found to have distinctive characters, *viz.*—

Strain A . . . On *santara* from Renala Khurd (culture No. 8·9/7)
Strain B . . . On *malta* from Lahore gardens (culture No. 1·9/24)
Strain D . . . On *malta* from Lahore gardens (culture No. 1·9/15)
Strain F . . . On *santara* from Lahore gardens (culture No. 15·8/19)

The above strains have been grown on oatmeal, rice agar and Czapek's agar, and their growth characters at 26°C. are given in Table IV.

TABLE IV

Growth character of four strains of C. gloeosporioides *on different media*

Strains	Oatmeal agar	Ricemeal agar	Czapeks' agar
Strain A. No. 316.	Mycelial growth poor, many acervuli, almost colourless, appear a little late. Spread 70 mm. in 7 days.	Very scanty colourless hyphæ at first—later blackish—central portion fluffy; black radiating hyphæ when viewed from below. Big sized hyphal knots seen. Spread 70 mm. in 7 days.	White fluffy growth; bottom—pink to dark gradually; central dark portion with plenty of acervuli. Spread 70 mm. in 7 days.
Strain B. No. 345.	Growth poor but largest number of minute acervuli formed. They appear early and develop in concentric rings. Clear from below. Spread 71 mm.	Growth whitish with dark hyphæ radiating in between (saltating ?). No acervuli in 7 days. Spread 66 mm.	Very white fluffy growth; Viewed from below appears blackish. Acervuli formed late, spread 70 mm.
Strain D. No. 347.	Growth poor but large number of acervuli formed later. Spread 71 mm.	Growth scanty—blackish radiating hyphæ seen when viewed from below; small hyphal knots. No acervuli in 7 days, but formed very late. Spread 65 mm.	White fluffy growth. Acervuli appear very late. Spread 72 mm.
Strain F. No. 233.	Cottony hyphæ; only a few acervuli developed. Whitish from below. Spread 72 mm.	White cottony growth; no radiating blackish hyphæ seen from below; only a few scattered acervuli formed very late. Spread 63 mm.	Fluffy white radiating ;. bottom pinkish to dark No acervuli in a week. Spread 73 mm.

From the above it will be seen that though the media determine the character of the growth to a great extent, the above four strains retain their distinctness in all these media, and may be taken as different strains.

(*f*) *Effect of exposure to low temperature*

Effect of very low temperature on the four different strains was observed. Five-day old cultures in oatmeal, rice and Czapek's agar in duplicate of each strain were placed in a refrigerator at 0°C. for a period of one month and twenty

days. Then these were transferred to an incubator at 25°C. All these revived. The causal organisms are not killed by prolonged exposure to very low temperature.

(*g*) *Heterothallism in* C. gloeosporioides

All the cultures A to L have been grown in groups in different Petri dishes to see how the two cultures behave when the hyphæ of one come in contact with the hyphæ of another, *viz.*, whether they mix together or form a space of aversion or a white line of aversion.

In all these cases monospore cultures have been used as stated before, and it has been noticed that certain cultures mix freely when put together, in others a space of aversion is formed between the two, while in some other cases a white line of aversion is formed. These results show definitely heterothallism in this fungus. Though all combinations have been tried, no fruit-bodies have been made to form yet. Complete fusions were seen between E-C, F-D, B-D, B-H, J-K, F-C, B-H, E-G.

Space of aversion between B-E, A-G, G-B, E-A, A-D, K-L, L-I, L-J, C-D, A-C, C-D, H-C, C-G, C-H, B-F, D-F, A-G.

White line of aversion between B-F, A-B, C-E, A-B, A-F, G-F, B-D, C-E.

In Plate XVII, figs. 5, 6 complete fusions, spaces of aversion and white lines of aversion between certain strains are shown.

(*h*) *Method of penetration*

The method of infection by *C. gloeospo rioides* hasbeen studied on *malta* leaves. Spore suspensions were placed on the upper surface of the leaves. The spores on germination produce appressoria-like structures which stick fast to the surface of the leaves. These appressoria are very regular in shape and size and are able to withstand desiccation and should be looked upon as chlamydospores. The appressorium in *Gloeosporium* has also been described as a chlamydospore by Biraghi [1934]. When the spores germinate, they give out certain products which react on the cuticle and the plasma membrane and the cells below get injected before actual penetration takes place. The stimulus to germination of the appressoria might be supplied by nutrient substances diffusing out when cells get injected. Injection has been produced by placing filtered drops in which spores were germinating for thirty to forty hours and when later the leaf was flooded with a dilute solution of erythrosine, the dye was absorbed by the injected spots only. No fine peg-like infection hyphæ as noticed by Dey [1933] have been noticed but instead ordinary germ-tube like structures have been seen to penetrate.

The details regarding the method of penetration have been published by the author in a separate paper [Chaudhuri, 1935].

(*i*) *Pathogenicity*

Cultures of wither-tip organisms from *khatta* (*C. aurantium*), *malta* (*C. sinensis*) and *santara* (*C. poonensis*), as well as spores from nature were used for inoculation experiments. In the first series of experiments only cut leaves were used. These were washed in 0·01 per cent mercuric chloride solution, and after carefully washing in several changes of distilled water, were placed in sterilised Petri dishes containing moist cotton wool. These were now sprayed with spore suspension and incubated at 27°C. Table V shows the result of experiment after a week's observation.

TABLE V

Result of inoculation of malta, santara *and* khatta *leaves with* C. gloeosporioides *spores found on those host-plants as well as with spores formed in culture from them*

Fungus strain	Host	No. of leaves sprayed	No. taking infection	No. of control	No. of control infected	Remarks
Malta wither-tip spores from culture	*Malta* leaves	6	6	2	*nil*	Many acervuli on upper surface of inoculated leaves.
Do. spores naturally formed	Do.	6	6	2	*nil*	Several spots with acervuli on upper surface of inoculated leaves.
Santara wither-tip spores from culture	*Santara* leaves	6	6	2	*nil*	Brownish spots, white mycelial growth, acervuli on both sides of inoculated leaves.
Do. spores naturally formed	Do.	6	6	2	*nil*	As above, acervuli bursting.
Khatta wither-tip spores from culture	*Khatta* leaves	6	6	2	*nil*	Brownish spots on inoculated leaves but no acervuli formed yet.
Do. spores naturally formed	Do.	6	6	2	*nil*	As above

Plate XVI, fig. 4 shows innumerable acervuli formed on a *malta* leaf when sprayed with *malta* wither-tip spore suspension in distilled water.

When the above experiment was performed at 15°C. instead of at 27°C., no change was visible for a week but when the Petri dishes were removed after that to a higher temperature (27°C.) quick changes were observed. Three days after removal to a higher temperature, positive infection was found on *malta* and *santara* leaves (whether sprayed with naturally formed spores or with spores formed in culture) but no infection was found on *khatta* leaves. Control leaves were all healthy. Table VI shows the results of cross-inoculation experiments with cut leaves in a tabulated form. Certain marked spots on the leaves were pricked with a sterilised lancet needle after the leaves had been washed in 0·01 per cent mercuric chloride solution and distilled water. Cultures of *malta* (N. S. 235), *pomelo* (N. S. 237), *santara* (N. S. 233) and orange (*Citrus aurantium* C. S. 233) wither-tip have been used. The experiments were all done at room temperature (25°—30°C.).

TABLE VI

Result of experiment using cut leaves

Host	Observation after		Remarks
	8 days	12 days	
(*a*) *Inoculated with spores from* malta *wither-tip* (*N. S. 235*)			
Orange (Assam)	Spots pale green .	Spots pale brown	No acervuli formed. No infection.
Khatta . .	No change . .	Spots pale yellow	Acervuli formed. Positive infection.
Pomelo . .	Spots pale brown	Spots pale brown	No acervuli. No infection.
Malta . .	No change . .	Spots yellowish brown.	Acervuli formed, some bursting, positive infection.
Lemon . .	Spots pale brown	Spots pale brown	Many acervuli, some bursting, positive infection.
Mitha . .	Spots pale brown	Spots pale brown.	A few acervuli formed, positive infection.
Galgal . .	Spots pale yellow.	Spots brownish .	No acervuli, no infection.

Host	Observation after		Remarks
	8 days	12 days	
(*b*) *Inoculated with spores from* pomelo (*N. S. 237*)			
Mitha . .	Spots brownish .	Spots dark brown	No acervuli formed, no infection.
Malta . .	Spots pale brown	Spots deep brown	Pinkish spore masses coming out of acervuli. Positive infection.
Santara . .	Spots pale green .	Spots yellowish .	Acervuli formed, positive infection.
Galgal . .	As above . .	As above . .	As above
Khatta . .	As above . .	As above . .	As above
Lemon . .	As above . .	As above . .	As above
Pomelo . .	As above . .	As above . .	Innumerable acervuli formed, positive infection.
(*c*) *Inoculated with spores from* santara (*N. S. 233*)			
Santara . .	Spots pale green .	Spots pale brown	Innumerable acervuli from which pinkish spore masses are oozing out, positive infection.
Orange (Assam)	Spots pale green .	Spots pale green .	No acervuli, no infection.
Pomelo . .	Spots pale green .	Spots dark brown	Acervuli formed.
Mitha	Spots pale green .	Spots pale brown	No acervuli formed, no infection.
Galgal . .	Spots pale brown	Spots dark brown	Acervuli oozing out, pink spore masses early. Positive infection.
Malta . .	Spots pale green .	Spots brownish .	Many acervuli, pinkish spore masses oozing out, positive infection.
Khatta . .	Spots pale brown	Spots dark brown	Acervuli formed, spore masses oozing out, positive infection.

Host	Observation		Remarks
	8 days	12 days	
(d) Inoculated with spores from orange (C. S. 233)			
C. trifoliata .	Spots pale brown	Spots dark brown	No acervuli, no infection.
Orange (Assam)	Spots pale yellow.	Spots brownish .	As above
Galgal . .	Spots pale brown	Spots dark brown	A few acervuli, positive infection.
Pomelo . .	Spots pale brown.	Spots dark brown	Acervuli formed, positive infection.
Mitha . .	Spots pale brown.	Spots brownish .	No acervuli, no infection.
Khatta . .	Spots pale brown.	Spots dark brown	A few acervuli, positive infection.

Table VII shows the result of inoculation with cultures isolated from wither-tip of orange (233 C. S. and 216 C. S.), *malta* (235 C. S.), *pomelo* (238 C. S.) and from a local *malta* leaf (C. S. 230) on different varieties of *malta*. The twigs were obtained from the Indian Mildura Fruit Farm, Renala Khurd.

TABLE VII

Result of inoculation of different varieties of malta *with spores from cultures isolated from different host-plants*

Varieties of *malta*	*Orange* 233 C. S.	*Malta* 235 C. S.	*Orange* leaf 216 C. S.	*Malta* leaf 230 C. S.	*Pomelo* 238 C. S.
	Acervuli				
Blood Red . . .	—	+	—	—	+
Wilkie . . .	—	+	—	—	+
Excellence . . .	—	+	+	—	+
Italian . . .	+	+	—	—	++
Jaffa . . .	+	+	—	—	+
Musumbi . . .	+	+	Few +	Few +	—
Ruby . . .	—	+	Few +	Few +	++
Washington . . .	—	++	—	+	—

In Table VIII, the result of inoculation of potted *malta* and *s ant* plants with two different cultures of *malta* anthracnose and one of *santara* has been shown. Four plants were inoculated with each of these strains and two plants of each were kept as controls. The plants to be inoculated were pricked at several marked places (not on the leaves) near the tip and then sprayed with a thick suspension of spores. Shedding of leaves began after ten days and in a fortnight twigs showed signs of wither-tip. Control plants were all healthy.

TABLE VIII

Result of inoculation of potted plants with three different strains of C. gloeosporioides

Fungus strain	Host	No. of plants inoculated	Observation after two months
Malta wither-tip culture 22·7/7 of 347	*Malta* .	4	Three altogether dead, one shows disease.
Do.	*Santara* .	4	All leaves shed, tips show disease.
Malta wither-tip culture 19·7/1 of 345	*Malta* .	4	One dead, two twigs dying back showing wither-tip, one healthy.
Do. .	*Santara* .	4	Every twig shows wither-tip.
Santara wither-tip culture 22·7/6 of 346	*Malta* .	4	One dead, two diseased, one healthy.
Do. .	*Santara* .	4	Three very badly diseased, almost dead, one slightly affect.

From the above experiment it is clear, that the strains from *santara* and *malta* inter-cross and pass easily from one into the other. Plate XVI, fig. 3, shows a potted *malta* plant which had been inoculated with a strain *C. gloeosporioides* from *santara*. Two of the branches are dead.

(*j*) *Effect of stocks on scions in producing resistance or otherwise if any*

In the plantations, in the Punjab various grafted plants of *malta* and *santara* are used. Generally the following are used in the Punjab as stocks :—*Khatta, khatti, pomelo, mokri, mitha, kimb, Citrus commerce, turang, Citron pickle* and *Eureka lemon*. In his trial of stocks at Peshawar, Brown [1920] used *khatti, khatta, mitha* and *galgal* as stocks, as these are mostly used by the local nursery men as their stock.

The culture Nos. 345, 346 and 347 were used in most of the inoculation experiments, as these strains were found to be virulent, and inoculated to the *malta* and *santara* leaves. In the following experiments cut twigs from grafted plants were taken and the effect of the stock on the scions on disease resistance or otherwise if any was studied. In one series the twigs were simply sprayed with spore-suspensions in distilled water and in the other before spraying incisions with a lancet needle were made on the twigs near the growing points. *Malta* twigs from grafted plants growing on the following stocks were taken—*Mokri, turang, Citron commerce, khatti, kimb, khatta, mitha, chakotra* and *Eureka lemon*. The experiments were carried out in early April and two twigs of each in each series were inoculated. The twigs were placed in small beakers half full of sterilised tap water and left in a glass chamber with special ventilation arrangement. The leaves of the infected twigs began to fall in four-five days' time. The tip became pale brown to dark brown and in seven-eight days' time acervuli were formed. When tips were infected, there was clear demarcation between healthy and diseased tissue as characteristic of the disease.

Controls were sprayed with distilled water and these remained healthy throughout the period of experiment. On the result of these experiments, it has been found that *Eureka lemon, khatti, turang* and *kimb* as stocks have some effect on the *malta* scions in producing disease resistance to a certain extent.

In the next series of experiments only the cut leaves of *malta* growing on different stocks were inoculated by spraying with spore suspensions after incision in one set and without incision in another. Three leaves were put inside sterilised Petri dishes containing soaked cotton wool. Controls were kept and were sprayed with distilled water, and these remained healthy throughout the period of eight days during which observation was made. It has been found that under very moist conditions and in a bad state of ventilation as inside the covered glass basins—*khatti, turang,* and *kimb* which were found in the previous experiment resistant to some degree, became susceptible. *Eureka lemon* however was found to be resistant to a higher degree.

Extensive field trials could not be done for want of suitable experimental orchards but in a small scale was carried out in the Indian Mildura Fruit Farm Orchard at Renala through the courtesy of the proprietors. Culture No. 346 from *santara* and No. 347 from *malta* were inoculated by means of pricking and spraying on the following varieties of *malta, viz.*, Blood Red, Valentine late, and common *malta* grafted on *kimb, Eureka lemon* and *khatta* stocks respectively. Five plants of each were inoculated in early September, immediately after the rainy season in the Punjab. The plants were under observation for over six months. Though Blood Red has been shown to be a susceptible variety by inoculation experiments, it was found that with *kimb* as stock no infection took place. Similarly Valentine *malta* on *Eureka lemon* remained free from disease. Only the common *malta* on *khatta* showed typical wither-tip.

From the results of the above experiments done in the laboratory and in the field, it may be recommended that *khatti*, *turang*, *kimb* and *Eureka lemon* should be used as stocks when making grafts for *malta* and *santara*.

Conclusion and summary

A major disease of the *Citrus* in the Punjab, *viz.*, wither-tip caused by *Colletotrichum gloeosporioides* Penz. has been studied. It occurs in an epidemic form in many places on *santara* (*C. poonensis*) and *malta* (*C. sinensis*). Though the disease is found throughout the province, it has been noticed that orchards situated near the foot of the hills suffer most. Beside withering of tips, great loss is caused by dropping off of immature fruits. Young plants may be killed outright. The diseased twigs turn silvery grey and become sharply demarcated from the healthy portion. The affected twigs and leaves bear minute dark acervuli around which dark septate or continuous setae are frequently seen.

Studies include growth of the fungus in culture, isolation of different strains and experiments regarding sex in *C. gloeosporioides*, method of infection and penetration, contributory causes, resistance of different species and effect of stock on scions (spraying tests and effect of manuring and aeration are described in the last chapter under control measures).

C. gloeosporioides grows well on various media in culture and the spread of the fungus, spore sizes and various growth characteristics at different temperatures have been given. The effect of concentration of media on growth has also been given. Several strains, showing not only difference in spore-measurements but permanent differences in growth characters, have been isolated and their virulence in causing disease has been tested. Presence of different sex characters in the different monosporous cultures has been found. When two cultures were grown side by side, in some cases complete fusions were seen, in others space of aversion was formed, while others had the white line of aversion. No fruit bodies however have been formed.

Though healthy plants have been successfully inoculated, it has been found that some of the severest injuries in connection with the disease may be due to the combined effect with other fungi.

Regarding contributory causes, it has been found that under unfavourable soil and climatic conditions or when the trees are injured by hail-storms or by any other means, the causal organism grows vigorously and quickly kills the plants.

With regard to the penetration of the organism into the leaf, it has been shown that the spores on germination in presence of water, give out certain products which react upon the cell walls and as a result the cells below always get injected before actual penetration takes place. Even when a filtered drop of water, in which spores were growing for twenty-four hours at 25°C., was placed on the upper surface of the leaf, injection of the cells below was produced. The infection tube

from the appressoria-like structures produced at the tips of the germ-tubes easily penetrate and enter into the cells below. It has been suggested that nutrient substances diffuse out from the injected cells and stimulate the appressoria to germinate.

For studying the resistance of different species of *Citrus* to the wither-tip, *khatta*, *khatti*, *malta*, *santara*, *galgal*, lemon, and *pomelo* leaves were separately inoculated with two strains of *C. gloeosporioides* which were found to be very virulent. As a result of inoculation experiments, it was found that under very moist conditions infection never failed but under comparatively dry conditions, some of the above plants showed resistance to a certain degree.

Different varieties of *malta* were similarly tested, but no resistant variety was found.

Regarding the effect of stock on scion, it was found that Blood Red *malta* which was proved to be a susceptible variety, was found to be somewhat resistant to wither-tip when grafted on *kimb* as a stock. Similarly the Valentine late or *Eureka lemon* remained free. Only the plants with *khatta* as stocks developed typical wither-tip symptoms. From the results of experiments done in the laboratory and in the field *khatti*, *turang*, *kimb* and *Eureka lemon* have been recommended as suitable stocks for *malta* and *santara* plants.

III. Other diseases of Citrus

(B) Chlorosis

(*a*) *External symptoms*

The leaves show a mottled appearance and drop down early. Plate XIII shows drawings of a few chlorotic leaves of *malta*. Chlorosis is also seen in *santara* but not so commonly as in the other species. When a tree becomes affected, only a few leaves or a few branches may show the symptoms, though in some cases all the leaves may be involved. In the individual leaves, the lighter areas may be confined only to a few spots or almost the whole of the leaf surface may become chlorotic. In the latter case only narrow green bands may be seen along the mid rib and base of the secondary veins, the whole of the remaining area turning pale green or yellow. The yield of fruits decreases considerably and the fruits become smaller and from badly affected trees, no fruits are obtained. In Valencia, in Spain, Regueral [1932] found considerable reduction of yield from " foliocelosis " (chlorosis) and he mentions general stunting of the branches and elongation of leaves in chlorosis. In Italy, Petri [1926] observed mottling in orchards badly affected with " wither-tip " (*Colletotrichum gloeosporioides* Penz.) but he found no connection between the fungus and chlorosis. Here in the Punjab, chlorosis has been noticed in *malta* plants absolutely free from " wither-tip " ; hence there can be no connection with the fungus, chlorosis being really

due to some sort of physiological trouble. Petri [1931] has also descri an infectious mottling of the leaves of *Citrus vulgaris* and, though he has not done any experimental work, he considers from the spreading nature of the disease, that it is carried by insect vectors, *viz.*, *Citrus* aphids which are always present. It may be mentioned here that in the Dhupsari plots of the Lawrence Gardens, Lahore, where the affected *malta* trees have been under constant observation, the *malta* plants are openly laid out and are free from *Citrus* aphids. In this plot plants show chlorosis at random. The chlorosis here is not thought to be of an infectious nature.

(*b*) *Internal structure and chemical nature of the chlorotic regions of the leaves*

Sections of chlorotic and contiguous green portions of the leaves were cut and examined. It was noticed that in the chlorotic portions, the pigments become light coloured and as the chlorotic portions turn yellow and white, the pigments also become gradually separated from the plastids and degenerate and in whiter portions, the plastids, specially of the inner palisade and spongy tissue, degenerate and empty cells may be seen collapsing. Somewhat similar symptoms have been observed by Petri [1931] in what he describes as infective chlorosis. In sections of the chlorotic portion of the leaves, it is a common thing to find the light green pigments which break down forming minute granules, to be seen in a state of motion.

Regarding chemical reaction of the chlorotic portions, Petri [1926] found that an excess of acid accumulates in the chlorotic region as compared with a contiguous green area. Sometimes it has been suggested that deficiency of calcium in the soil produces this condition. Regueral [1932] recommends application of calcium to stop this malady. But it has been shown that no correlation could be found between soil acidity or alkalinity and chlorosis. In Lahore, the soil from below the trees in the observed plants was tested, but no lack of calcium was noted, the pH value being 8·8. The soil from below the trees in different places showing chlorosis was also similarly tested and found to vary from 8·2 to 9·0 pH. In no case any dearth of calcium was noted. The pH value of the normal green and contiguous chlorotic portions of the leaves has also been tested. By means of a fine pair of scissors the different areas were cut out and the sap from the normal green and chlorotic areas were extracted separately by pressing in a vice between two thick ebony plates. As noted by Petri [1926] the chlorotic portions showed greater acidity. A large number of extractions was tested and the pH value of the chlorotic sap was found to lie between 6·4 and 6·6 and those of the normal green portions between 7·0 and 7·2. It may be mentioned that the pH was tested in early May when the leaves were comparatively young, and perhaps more acid might accumulate later when the leaves became older. This will again be done, but no correlation between accumulation of acid in the chlorotic regions and lack or excess of calcium in the soil could be made out in any case.

(c) *Experiments on the control of chlorosis of* malta

The injections of chemicals into plants for the control of fungous and bacterial diseases have been tried by various workers from time to time, but the results have been conflicting. This is apparently due to the fact that a solution of any chemical that will injure the protoplasm of the causal organism will also injure the protoplasm of the host-plant cells. Recently the injection of iron salts into the trunks of trees for combating chlorosis of various plants has received considerable attention. Bennet [1927] effected a ninety per cent cure of chlorosis of pear trees by injection of iron salts, and Wann [1930] by placing dry salts of ferric ammonium in holes bored into the stem of grape vines, peach and apple trees effected a similar cure ; the best response, however, was obtained by him from ferric phosphate. Thomas and Haas [1928] checked chlorosis of orange trees by injection of iron salts but they found subsequent injections necessary, since the wood was injured for some distance along the point of injection. A short note on the curative value of iron salts when injected has been published by the author [Chaudhuri, 1933]. In the experiments for the cure of the chlorotic condition of the *malta* leaves carried out here, the efficacy of different iron salts has only been tried. These salts have not only been injected but also sprayed over the plants with beneficial results. The experiments were carried out in early November with chlorotic *malta* plants only. In the preliminary experiment, cut twigs of *malta* bearing chlorotic leaves were placed in 0·01, 0·001 and 0·0001 per cent of the following salts : Ferric phosphate, ferrous phosphate, ferric sulphate, ferrous sulphate and iron-alum. Control twigs were placed in tap water. These were kept under observation for seven days in a big glass chamber, the atmosphere being naturally much more humid than that outside. It was observed that in solutions 0·01 and 0·001 per cent all the twigs except those in iron-alum became dead in three days. The leaves of the chlorotic control twigs also dropped down and in four-five days, the leaves of the non-chlorotic control twigs also dropped down. In iron-alum, 0·01 per cent solution, it was observed that the twigs remained very fresh for over seven days but no recovery from chlorosis was noticed and in dilute solutions of iron-alum, the leaves dropped down in three-four days. The other iron salts, however, proved beneficial in very dilute solutions (0·0001 per cent), and partial recovery from chlorosis was specially noticed in ferrous sulphate and ferrous phosphate solutions.

In the second series of experiments, cut twigs with chlorotic leaves were first placed in normal culture solutions. After twenty-four hours these were removed and placed in 0·0001 per cent of the different ferric and ferrous salts for twelve hours and then were replaced in the culture solution. Similar results were observed. The efficacy of the salt solutions in 0·0001 per cent solutions may be put in the following order :—

1. Ferrous sulphate—most helpful, many recoveries.
2. Ferrous phosphate—helpful, partial recovery.
3. Ferric phosphate and ferric sulphate—very slight effect, if any.
4. Iron-alum—no recovery.

Simultaneously with the above experiment, in another set detached chlorotic *malta* leaves were placed in large glass basins with cover over soaked cotton wool and sprayed with 0·0001 per cent solutions of the above salts. Partial recovery was noticed only with ferrous sulphate and ferrous phosphate solutions. Hence these two were selected for field experiments.

Solutions of these salts, 0·0001 per cent, were made and 2·3 c.c. were introduced very slowly at a time by means of a hypodermic syringe. Two injections were made in each twig. After three days the twigs injected with ferrous sulphate solution showed definite recovery. Simultaneously, when some twigs were injected, other chlorotic plants were profusely sprayed with ferrous sulphate and also with ferrous phosphate solutions ; these showed recovery. The new leaves that developed in plants sprayed with ferrous sulphate showed freedom from mottling, though in the following year the plants again formed chlorotic leaves. There can, however, be no doubt that ferrous sulphate in 0·0001 per cent solution helps recovery from chlorotic conditior and as injection method requires special care and technical skill, spraying with ferrous sulphate is recommended and spraying twice in the year will no doubt be found useful in curing chlorotic condition of *malta* trees.

Summary

Chlorosis of *malta* (*Citrus sinensis* Grove) has been investigated. Chlorotic areas become slightly more acid than the adjoining green portions. No correlation with the soil condition has been found. In transverse sections the chlorotic areas show light green chloroplastids and in bad cases where the regions turn yellow or whitish, break-down of the granules is seen and also the plastids degenerate and the cells become empty and collapse. Injections and spraying with different iron salts have been tried for curing chlorotic condition. Ferrous sulphate in 0·0001 per cent solution, whether injected or sprayed, has been found to be most efficacious and as spraying requires no special skill, this method has been recommended.

(C) Saprophytic moulds

(*a*) *Symptoms*

These are found to a certain extent in almost every garden, but in certain orchards they appear in a severe form and practically cover all the leaves (Plate XIV) and even fruits with a black incrustation, consisting of a weft of black mycelial threads of fungi of more than one species usually. When complete incrustations are formed, the sunlight is to a great extent cut off and consequently normal physiological processes of the leaves are interfered with. Not only does less formation of starches and sugars so necessary to the growth of the plant take place, but it has been observed that the fruits so covered have a sour and fermented

taste. Often the yield becomes very much reduced. Where fruits are covered in patches by the sooty mould incrustations, they develop a mottled appearance which is seen on cleaning the fruits. This is due to the fact that the covered patches do not ripen with the rest of the fruit surface and these retain a light green colour. Sometimes leaves covered with sooty mould curl and shrivel up under dry conditions. The black covering of sooty mould is, however, entirely superficial and may easily be rubbed off. Under dry conditions, they themselves become detached and are blown off in bits by the wind. They do not enter into the tissues of the leaves, but live on the honey-dew secreted by the scale insects in the wake of which they follow, and the control of the disease depends to a great extent on the control of the insects, for without their excretions, they will soon disappear.

Abundance of sooty moulds is generally found in the orchards where the trees are very closely planted or where the trees are shaded by other tall trees around them, so that light is interfered with and the atmosphere remains more humid. The following fungi have been found to take part in forming the sooty incrustations, *viz.*, *Acrothecium lunatum*, *Capnodium citri*, *Alternaria citri*, *Cladosporium herbarum* var. *citricola* and *Chaetomium* sp. The ascigeral fungus, *Pleospora herbarum*, has also been isolated. Any one of the first four species may predominate. Composition differs in different places, perhaps also in different seasons. The presence of *Alternaria* in sooty moulds may lead to infection of the fruits at the stylar end causing black rot of oranges and also dropping of immature fruits. *Acrothecium* may also produce a similar black rot of the fruits if it can gain entrance.

(*b*) *Acrothecium lunatum* Wak.

It has been isolated from the black incrustation on the leaves and grown in culture on various media. The conidia are curved, knee-shaped, 2-4-septate with large middle curved cell (Plate XVIII, fig. 2). It measures $18{\cdot}22 \times 5{\cdot}7\ \mu$. It grows well on a large variety of media. On starchy media like potato agar, pea agar, bean agar, etc., greater development of aerial hyphæ takes place but the spores are usually smaller, whereas, in synthetic media like Raulin's, etc., lesser development of aerial hyphæ takes place but the spores are bigger. It has been grown on various concentration of Raulin's. In higher concentrations, no spore formation took place and the mycelium developed a slight pink colour and in very low concentrations ($N/32$), scanty development of hyphæ took place and the growth was zonal. Regarding width of the spores it was found that the mean breadth from N to $N/32$ concentration decreased gradually from $6{\cdot}9\mu$ to $5{\cdot}2\mu$ and the area of spores (length × breadth) also uniformly decreased from normal to $N/32$ concentrations, though the greatest mean length of the spores was found at $N/16$. In hanging drops, the spores germinate readily. Germ-tubes are ormed either from one end or both ends. Inoculation experiments have also

been done. Pug inoculations were made. The inoculum was introduced inside both *malta* and *santara* fruits. In a week's time when the fruits were cut, jet black rot was seen. The fungus was re-isolated from the inoculated fruits. Control lies in removing the source of contamination by spraying the leaves with fungicides.

(*c*) *Capnodium citri* Berk. et Desm.

This fungus (Plate XVIII, figs. 1-2) lives entirely superficially on the leaves and fruits of *Citrus* sp. on the excretions left by the scale insects. In *Citrus*, it never gets inside the tissues and causes no rot. In certain regions and in certain seasons, this forms the predominating sooty mould fungus. Fawcett and Lee [1926] mention that "when it occurs in abundance, it may seriously retard the growth, causing light blooming and flowering, and rendering the tree less resistant to drought." The curling and shrivelling of leaves affected by sooty mould under dry conditions has already been mentioned. The colour of the hyphæ varies from olive-green to deep brown, and they form a dark brown crust on the surface. This fungus reproduces by conidia, pycnidia, stylospores and perithecia, and these have been described and drawn in detail by Webber [1897]. The measurement of the different kinds of spores varies a lot and they grow readily in cultures. Conidia, pycnidia and stylospores are abundantly formed in cultures, but their shapes and sizes differ very much. Stellate spores of *Triposporium* form are readily formed from the hyphæ (Plate XVIII, fig. 2). These germinate readily. Control by spraying with fungicides.

(*d*) *Alternaria citri* Pierce

It is almost always associated with the sooty mould fungi and may cause dropping of fruits and also a black dry rot of the fruits if it gets inside the fruits. In many parts of the Punjab, *Alternaria* rot is common. It gets in either through the stylar end or stem end of the fruits which gradually becomes pale and discoloured. *Alternaria* can only get in through some injured tissue produced either mechanically or through insects. The rot is seen when the fruit is cut open. *Alternaria citri** has been isolated both from the sooty-mould incrustations on the leaves and fruits and also from the rot inside the fruit. The culture from the mould as well as from the fruit rot have been grown side by side in various media and have been found to be identical. This fungus grows readily in almost any media and the spores which are septate are in chains (Plate XVIII, fig. 4). These spores also germinate readily, and the germ-tubes may be formed from the two end cells or even from each cell of the spore. The measurement of the

*As *Alternaria* spores are almost always present, care should be taken when picking the fruits, otherwise through the least injury, the fungus will get in and produce rot in storage.

spores in various media shows that they differ a good deal. Effect of different concentrations of Richards' medium on growth and spore formation was studied. *Alternaria* grew very well at 8*N* concentration when the largest size spores were also formed. Inoculation experiments have been made and it has been found that it is a wound parasite. Plug inoculation of fruits produced the characteristic rot. As the source of contamination is always present in the sooty moulds, spraying with fungicides to remove them will prove effective in checking it.

(*e*) *Cladosporium herbarum* var. *citricola* F. & B.

It is very commonly found in the sooty-mould incrustations of the *Citrus* leaves. This fungus also causes what is known as "leprosis" or "nail head rust" of sweet oranges. It produces sunken spots on the rind. The fungus readily grows in culture and abundant spores are formed (Plate XVIII, fig. 5). The spores vary in shape and size (8-12·6μ × 2·7-3·1μ). In culture, the fungus develops a greenish tinge.

(*f*) *Chaetomium* sp.

Sometimes it forms an important fungus in the sooty-mould incrustations. It is a purely superficial fungus like the *Capnodium* living on the excretions of the scale-insects. It forms dark black hyphæ and perithecia with asci are often seen and are formed readily in cultures (Plate XVIII, fig. 7). It may readily be removed by spray mixtures.

(*g*) *Pleospora herbarum* Rab.

It may be looked upon as quite a common black mould of *Citrus* leaves in many parts. Perithecia formed on almost any media—even on moist cotton wool plenty of fruit bodies are formed. Ascospores germinate readily (Plate XVIII, fig. 6). Other species of *Pleospora* are found on dead twigs of *Citrus* forming fructifications.

Conclusion and summary

Organisms taking part in the formation of sooty-mould incrustations of *Citrus* leaves and fruits in the Punjab have been isolated and their growth in culture studied. Though not much actual damage is done to the leaves by the sooty coverings, the fruits so covered turn bitter in taste. Besides some of the organisms taking part may cause important diseases and always remain as a perpetual source of infection unless properly controlled. For instance, *Alternaria citri* may cause considerable loss in conjunction with *C. gloeosporioides* by bringing about fall of immature fruits. Similarly *Cladosporium herbarum* var. *citricola* may cause leprosis or nail head rust of sweet oranges.

Six different fungi, *viz.*, *Acrothecium lunatum*, *Capnodium citri*, *Cladosporium herbarum* var. *citricola*, *Alternaria citri*, *Chaetomium* sp., and *Pleospora herbarum* have been commonly found to take part in the formation of sooty incrustations of *Citrus*. Most of these are known to occur on *Citrus* and other fruit trees in different countries. Preti [1923], Janini [1923], Tengwall [1924], Bolle [1924], Fraser [1933-1934] and others have investigated the composition, systematy and physiology of the fungi. Beside the forms noted above *Aspergillus* is also readily found in the sooty incrustations. All those moulds which follow in the wake of the scale-insects and live on their excretions, may be effectively controlled by controlling the scale-insects. Details regarding spraying for their control have been given in the chapter on control measures.

IV. Control measures

(*a*) *Control experiments at Lahore.*

Extensive experiments for the control of the disease in the field by means of spraying, manuring, aerating, etc., have been done, and will be described later in this chapter. First a few experiments to see the efficacy of certain spray mixtures in preventing the disease are described. In the preliminary experiments done in the laboratory only Bordeaux mixture (5 : 5 : 50) and two proprietory mixtures, *viz.*, Sulfinette and Arboretas Wash were used. The experimental plants were kept under observation for over two months and only disease-free plants were selected. These were first sprayed with the fungicides and were then sprayed with spore suspensions after a week.* After a fortnight all the *malta* and *santara* plants excepting those previously sprayed with Bordeaux showed positive infection. Plants sprayed with Sulfinette and Arboretas Wash, though infected, were no doubt in a much better condition than the unsprayed plants which were very badly infected.

Systematic spraying of fungicides for the control of wither-tip and other associated diseases was taken up from early November, 1931. At that time the plants were all bearing fruits and hence no pruning could be done. A very badly affected plot was selected in a garden (Mangal Singh's garden, near Shahdara) near Lahore. Almost all the twigs of the plants showed wither-tip (Plate XV) and every day a large number of immature fruits were dropping on account of infection by *Colletotrichum* and sometimes also on account of other organism. The affected fruits showed pale yellow colour, and from a large number of fruits the

* For the preparation of fungicides the following books have been freely consulted :—

Anderson and Roth	. Insecticides, fungicides and Appliances, 1923.
Bourcart and Burton	. Insecticides, fungicides and Weed Killers, 1926.
Mason . .	. Spraying and Dusting and Fumigating of Plants, 1929.
Fawcett and Lee .	. Citrus Diseases—Chapter on fungicides, etc., 1926.

causal organism was isolated. These fruits also become bitter in taste and rather sour. In this plot, the leaves of the plants were all covered by sooty moulds. Two different spray mixtures were tried, *viz.*, Bordeaux Paraffin oil emulsion and Sodium sulphate-kerosine oil emulsion. The following shows the result of spraying :—

Date of spraying	No. of trees sprayed	Spray mixture	Quantity sprayed	Remarks
2nd Nov. 1931	8 *mitha* . 1 *galgal* .	Sodium sulphate kerosine oil emulsion. Sodium sulphate 6 oz. Kerosine oil emulsion 650 c. c in 12 gall. water.	12 gall. .	Not very effective for fruit-fall but prevents sooty-mould.
4th Nov. 1931	30 *malta* .	Bordeaux Paraffin oil emulsion. Copper sulphate 4 lbs. Unslaked lime 4 lbs. Paraffin oil emulsion 1 gall. Water 49 gallons.	40 gall. .	Spray mixture spreads uniformly and sticks for a long period. After a fortnight fruit-fall checked. No further spread of disease.*

Four *mitha* and four *malta* were left untreated as control plants. In these the fruit-fall continued unchecked.

The Dupsari Citrus plot in Lawrence Garden, Lahore, where the plants were badly attacked by wither-tip and other diseases, was selected for testing the efficacy of the different spray mixtures, *viz.*, Bordeaux mixtures (4 : 4 : 50 and 5 : 5 : 50), Bordeaux with crude oil emulsion, Ammoniacal copper sulphate, Home-made lime sulphur and also the following proprietory fungicides, *viz.*, Arboretas Wash, Cooper's fungicide, and Sulfinette. As this plot was badly, infected, there were very few fruits on the trees. These trees were heavily pruned before first spraying was done. All cut twigs were carefully collected and burnt. The second spraying was done with the same spray mixtures on the same trees in middle of May, after new fruits had been formed. The spraying was always done in the afternoon. The details regarding spraying and the results are shown in Table IX.

* In the following March these plants were pruned heavily and sprayed once more before the flowers came out. Next season there was practically no loss from fruit-fall in that plot.

Table IX

Result of spraying of malta *trees with different fungicides. First spray as on date in the table, second one during middle of May 1932.*

Date of spraying	No. of trees	Spray mixture	Quantity sprayed	Remarks
			(Gallons)	
2nd Dec. 1931 .	12	Bordeaux (4 : 4 : 50).	10	No spray injury, many twigs dying back, on dead twigs fruiting bodies of *C. gloeosporioides* and also of *Gloeosporium* appear, leaves pale green in colour, fruits bigger.
3rd Dec. 1931 .	14	Sulfinette (1 in 40).	8	Young leaves were scorched and later all twigs and branches showed wither-tip progressing.
4th Dec. 1931 .	21	Cooper's fungicide (1 in 16).	10	These plants had no fruits, no spray injury but anthracnose spots appeared on leaves, later, twigs showed wither-tip.
10th Feb. 1932	52	Cooper's fungicide (1 in 16).	22	Results as above.
17th Feb. 1932 .	28	Sulfinette . .	12	Scorching seen, fruits sunburnt, twigs also showed wither-tip progressing.
Do .	38	Bordeaux(4 : 4 :50)	20	No injury, but disease not checked.
Do. .	18	Bordeaux(5 : 5 : 50)	10	No injury, better result than Bordeaux (4 : 4 : 50) some plants (5 out of 18) badly affected.
Do. .	15	Bordeaux and crude oil emulsion.	6	No injury, trees healthier, 5 out of 15 plants showed mild wither-tip.
Do. .	24	Ammoniacal copper carbonate.	17	No scorching and injury to fruits, disease held in check for some time, but later twigs showed wi- ther-tip.
Do. .	38	Home-made lime sulphur.	13	No injury, no scorching of fruits, similar in effect to Bordeaux (4 : 4 : 50).
Do. .	80	Arboretas wash .	7	The wash is perfectly safe to use, young leaves not scorched, trees have healthier appearance, disease checked, 22 *malta* out of 80 showed disease.

Half a dozen *malta* trees showing wither-tip were left untreated as control plants during the progress of the experiment. At the end of two years, these plants showed wither-tip right up to the stump—the upper portions being dead. These were then cut down and burnt. Regarding the efficacy of the different spray mixtures as seen from the above experiments it may be summed up that Bordeaux-oil emulsion and Bordeaux mixture (5 : 5 : 50) proved helpful and of the proprietory fungicides Arboretas Wash proved a useful one. The cost of spraying per tree per spray is given below :—

	Rs.	A.	P.
Ammoniacal copper carbonate	0	2	9
Arboretas Wash	0	1	0
Bordeaux-oil emulsion	0	0	9
Sulfinette	0	0	9
Home-made lime sulphur	0	0	5
Cooper's fungicide	0	0	3
Bordeaux mixture	0	0	3

In July 1932, the orchard adjoining the Superintendent's Bungalow, Lawrence Garden, Lahore, was chosen for spraying with fungicides. This plot contained mostly *malta* plants and all the plants, without exception, were affected by wither-tip. Three hundred and thirty plants were sprayed with 325 gallons of Bordeaux-crude oil emulsion. The cost per tree for spraying worked at nine pies per tree (including labour) as follows :—

	Rs.	A.	P.
Copper sulphate—16¼ seers	8	2	0
Lime—16¼ seers	0	8	0
Crude oil—1½ gall.	1	14	0
Soap—1 seer	0	8	0
Other charges (carrying spray machines, vats, etc.)	2	6	0
Total	13	6	0

At the time of spraying in July a large number of fruits were daily dropping down on account of the stem-end-rot caused by *C. gloeosporioides*. On dissecting the fruits the dry rot could be seen progressing through placenta into the pulp. Sometimes the fruits dried up and remained attached to the stalks. The stalks from the bottom end (2-in. to 3-in.) sometimes dried up and acervuli were seen developing on them.

As a result of spraying, further fruit-fall was checked, but the result on the whole was not as satisfactory as might be expected, for during the weeks following

spraying, there were frequent showers, which washed off much of the spray materials. Hence later on Bordeaux mixture with either ferrous sulphate as a sticker * or Rosin † for the same purpose was used. Rosin sticker was used in plots where plants suffered from sooty mould as well. Sometimes Bordeaux-rosin sticker with nicotine sulphate ‡ specially where scale-insects were present, were used. The use of the last-named spray mixture, however, is objected to by the Sikhs on religious grounds as it contains nicotine. So Bordeaux-rosin which is a cheap and effective fungicide is recommended for use. In many of the orchards chlorosis is a common form of ailment, and in those cases Bordeaux with a ferrous sulphate sticker has proved of great help (chlorosis and its control have been dealt with separately in another chapter).

In a private garden at Lahore seven *Citrus* (four *malta* and three *santara*) trees were very badly affected by wither-tip and bore branches which were killed 2 ft.-3 ft. downwards. The trees were almost dying. Leaves showed mottling. These were heavily pruned and sprayed with Bordeaux containing ferrous sulphate as a sticker (22/1/37). There was no injury to the leaves, and though it rained several times, the fungicide was not washed off (6/3). This fungicide proved very effective. The plants all revived, grew well, no wither-tip appeared and the leaves were no longer mottled but developed a healthy green colour. Next season, all these plants fruited very well.

In the laboratory, forty other *Citrus* plants (*malta*, *santara* and *mitha*) which were flowering at that time (7/3) and were full of scale insects, were sprayed with Bordeaux (3 : 3 : 50) with rosin-sticker. When examined after a fortnight the plants were found free from the aphids, and it was seen that there was practically no injury done to the flowers. In another lot of plants growing in pots and attacked by scale-insects, spraying was done with Bordeaux with Rosin-sticker and nicotine sulphate with very good results. To 12½ gallons of Bordeaux mixture (4 : 4 :50) 60 c.c. of nicotine sulphate (40 per cent) and 1,000 c.c. Rosin-sticker were added. This spray may be used in plants in which the fruits have set with very good result, but should not be used in flowering time. In the laboratory, six plants which were flowering at the time of spraying did not bear any fruits.

In July 1933, three hundred *Citrus* plants (mostly *malta*) in the Dupsari plot were sprayed with 350 gallons of Bordeaux mixture (5 : 5 : 50) with Rosin-sticker after pruning. These plants had been sprayed in the previous season. Three hundred and thirty plants in the orchard adjoining Superintenent's Bungalow, which had in the previous season been sprayed with Bordeaux mixture (5 : 5 : 50), were sprayed with 350 gallons of Bordeaux with ferrous sulphate as a sticker.

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* Copper sulphate 5 lbs., ferrous sulphate 4 lbs., lime 10 lbs., water 50 gallons.

† Rosin 2 lbs., Washing soda 1 lb., Water and Bordeaux mixture 50 gallons. (4 : 4 : 50).

‡ With Bordeaux-rosin (50 gall.), ½ pint of Nicotine sulphate (40 per cent) was used.

Both the above mixtures have proved very useful but the errous sulphate sticker sticks for a much longer time. Leaves of plants sprayed before the rainy season (in May) bore traces of the spray long after the rains (October). The former, *viz.*, the Bordeaux with Rosin-sticker costs seven pies per plant per spray and the latter six pies per plant per spray.

The plot adjoining the Superintendent's Bungalow was once more sprayed in February 1934, and this plot with over 300 plants may be said to be free from the disease. The yield of fruit last season was very high and there was practically no loss from fruit-fall.

(*b*) *Control experiments at Nurpur*

Nurpur is situated at the foot of the hills in the Kangra Valley. It is a very important centre for *Citrus* cultivation and in and around Nurpur, there are quite a number of large *Citrus* plantations. Sooty moulds heavily cover the leaves of most of the trees in this area and wither-tip disease has appeared in rather an epidemic form.

Through the courtesy of the Hon'ble Mr. Justice Tek Chand his *Citrus* orchard just opposite the Nurpur Railway Station was placed at our disposal for control experiments. Bordeaux mixture with Rosin-sticker was exclusively used. Sixteen hundred plants (*santara* 1,277, *malta* 150, *nimbu* 113 and *galgal* 60) were sprayed with 1,600 gallons of the spray at a cost of seven pies per tree (1 : 6 : 33), after these had been lightly pruned. The spray killed the sooty moulds which fell off in crusts*, and though rains started after a fortnight the spray was not completely washed off (29 : 8 : 37). The plants improved very much in appearance, and there was practically no loss from fruit-fall. When in the following February, the plants were heavily pruned and sprayed once more with the same fungicide there was very little of wither-tip or sooty mould. The plants fruited more plentifully. Most of the growers in that area took much interest in our work, and as they were convinced of the usefulness of these fungicides, many of them have taken up control measures according to our directions.

(*c*) *Recommendations for disease control by spraying of fungicides*

For the control of the wither-tip and the associated diseases, Bordeaux mixture (5 : 5 : 50) with ferrous sulphate as sticker, where sooty moulds are absent, or with Rosin as a sticker, where sooty moulds are present is strongly recommended for use. As the sticking power of ferrous sulphate is greater and as it checks mottling to a great extent, its use as an alternative spray mixture after using Rosin-sticker to remove and control the scale-insects is recommended. For plants which are badly attacked by the aphids, on the excretions of which the scale insects live, nicotine sulphate with Bordeaux-rosin may be used as a first spray, but it should never be used when plants are flowering.

* When these crusts were plated, the spores did not germinate.

In this connection a few words about quarantine treatment of imported plants for the prevention of disease will not be out of place. In India many nurserymen and private owners import *Citrus* plants and stocks from Australia and other places. At the Bombay Custom House, the plants are fumigated by hydrocyanic acid gas. To find out the efficacy of the treatment a packet containing *Citrus* twigs badly infected by wither-tip and *Citrus* canker, was made and sent to the Chemist at the Custom House at Bombay for fumigation. The packet was loosely wrapped in muslin. It was treated to three hours' exposure of about 0·07 grm. per cubic foot of hydrocyanic acid gas to start with. The concentration is only approximate, for as the Custom Chemist says, it is arrived at by calculation on the basis of the quantity of potassium cyanide used to generate hydrocyanic acid gas and the cubical contents of the fumigation chamber.

When the packet was received back in Lahore, thick spore suspension was made from the spores in acervuli and several platings on potato-agar were made and incubated at 25°C. After three days the plates were absolutely full of colonies of *C. gloeosporioides*. Plating of the canker organism from the treated material also showed that they were not in the least affected by three hours' treatment of hydrocyanic acid gas at that strength, and the number of colonies formed in both cases was similar to those from the untreated ones which were made for comparison.

(*d*) *Control of disease by manuring and aeration*

It has many a time been claimed that plants resist disease if they grow under a healthy condition inducing vigorous growth. In *Citrus* plants, in the Bombay Presidency, the die-back disease has been claimed to be successfully controlled by proper manuring and aeration [Sahasrabuddhe, 1927 ; Cheema and Bhatt, 1929]. To find out if vigorous growth could help the *Citrus* plants in checking the wither-tip disease, experiments on manuring and aeration and also the combined effect of the two have been done both in Lahore and in Nurpur ;

Only two kinds of manures, *viz.*, sulphate of ammonia and Niciphos Grade II (containing equal quantities of nitrogen and phosphoric acid, *viz.*, eighteen per cent nitrogen and eighteen per cent phosphoric acid) supplied by the Imperial Chemical Industries were tried. For applying the manure the soil is watered first and then a 6-in. to 9-in. deep groove is cut round the trees where the spread of the branch finishes. About two lbs. of manure is now put in the trenches round each tree and thoroughly mixed with the soil.

In Dupsari plot in the Lawrence Garden, Lahore, forty *malta* trees were treated with Niciphos and forty-four with sulphate of ammonia, twenty were untreated and kept as control and in the plot adjoining the Superintendent's Bungalow forty *malta* were treated with Niciphos and sixty with sulphate of ammonia, but in between the rows of plants (distance approximately 15 ft.) trenches two ft. wide and three ft. deep were dug. The trenches were cut a month previous to manuring the plants. Between these rows of untreated plants two similar trenches were

dug and two rows of plants were left untreated and without any trenches by their side. The trenches were dug at the end of July. It rained off and on throughout August and September and after three months, *i.e.*, by the end of October the trenches were filled up. In Nurpur Control Station, experiments with manuring only were made, but no trenches were dug. Manuring was done at the end of August. Thirty-two *santara* and twenty-six *malta* plants were treated with Niciphos and thirty-two *santara*, fifteen *malta* and thirty-two *galgal* plants were treated with sulphate of ammonia.

The cost of treating each plant with Niciphos was Rs. 0-3-3, and with sulphate of ammonia Rs. 0-2-2 only. The experimental plots in Lahore were visited every fortnight and the Nurpur Station every two months. It may be mentioned at the outset that no appreciable difference in the growth and vigour of plants was found by aeration only. Perhaps aeration might improve the condition of the plants in ill-drained soils. But manured plants showed distinct superiority over the unmanured ones, the newer leaves being of a deeper green colour, and there was greater abundance and bigger size of fruits the following season and in this respect Niciphos proved a better manure. As most of these plants had been under control spraying, the check of wither-tip in these plants could not be ascribed to manuring only. But a few plants which had not been sprayed but treated with manure, though improved very much in general condition, showed the presence of wither-tip as before. That manuring alone cannot prevent the disease is evident from the experiments carried out by the Imperial Chemical Industries themselves in the Government Botanical Garden, Saharanpur, where a new orchard treated regularly with different kinds of manures from the beginning, showed the presence of wither-tip and canker in many of the plants.

(*e*) *General observations*

To prevent the disease and to deal effectively with it when it has appeared, the orchardist must properly nourish his plants, prune the diseased twigs every season and burn those twigs and as a routine work spray once immediately after pruning (February-March) and once more late in September or early in October when the fruits are growing, according to the directions given already. Not only have the orchards to be maintained in a disease-free condition, but it is equally or perhaps more important to keep the nurseries in a sanitary condition for, as mentioned already, the diseases in the Punjab have in recent years been found to spread mostly from the nurseries which are very badly infected by wither-tip and canker. Owing to ignorance, people buy infected plants. For this, dissemination of knowledge about diseases in plants is essential. The author [Chaudhuri, 1936] published a scheme for this purpose. Growers should be advised on simple control methods, and nursery plants have to be sprayed regularly for protection against diseases ; also these have to be treated for disease if any appears. There should be arrangement for regular inspection of nurseries and granting of certi-

fication of health for nursery stocks by a competent authority and the certificate should be produced at the time of sale of plants. The public should be advised not to buy any plants from an uncertified nursery. For certification work, the teachers of plant pathology in the Universities could easily co-operate with the Government Agricultural departments. Thus not only the wither-tip disease of the *Citrus* but many other diseases could be effectively controlled.

Mention has already been made, in a previous chapter, about the encouraging results obtained regarding the effect of stock on scion in resisting wither-tip disease of *Citrus*.

Summary

Spraying tests, including trials of proprietory mixtures and the effect of manuring and aeration, are described.

Systematic spraying with the following fungicides for the control of wither-tip and other associated diseases was done in selected orchards in Lahore and Nurpur :—Bordeaux and Burgundy mixtures, Ammoniacal copper carbonate, Home-made lime sulphur, Sulfinette, Cooper's fungicide, Arboretas Wash, etc.

Bordeaux mixtures proved most effective but as it may often be washed away during rains, Bordeaux with ferrous sulphate as a sticker has been successfully used and it also helps to reduce chlorosis of the leaves found in many orchards. In many orchards sooty moulds cause trouble in addition to wither-tip and other diseases. Experiments with combined spray mixtures have given satisfactory results. Bordeaux-oil emulsion, Bordeaux with Rosin and Bordeaux-rosin with nicotine sulphate have given excellent result.

Spray mixtures should not only be cheap and easy to prepare but also must not contain ingredients which may be objectionable on religious grounds. Manuring and aerating improved the general conditions of the plants but did not prevent the disease.

Nursery attacks are very serious and control has been discussed under the following heads :—propaganda in nursery sanitation ; need for mycological control, certification of health for nursery stock and advice to growers on simple control methods.

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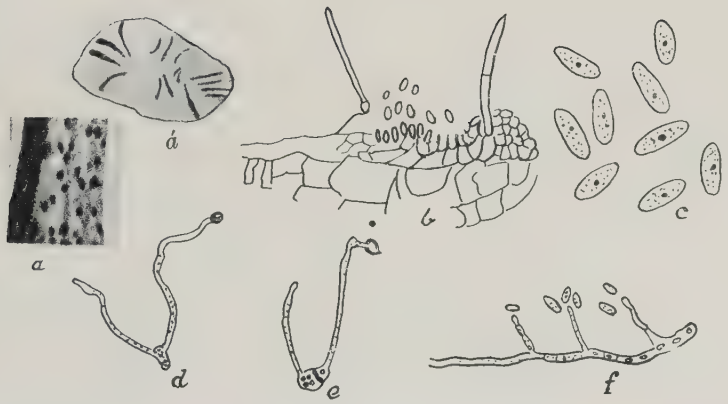


(*a*) Photograph of a twig of *malta* showing acervuli of *C. gloeosporioides* (slightly magnified). (*á*) acervulus with dark setae (surface view) (highly magnified). (*b*) Section through an acervulus (greatly magnified). (*c*) Spores (×650). (*d*), (*e*) Germination of spores. Note dark coloured appressoria produced (×350). (*f*) Spores formed in a hanging drop culture (×350).

A twig of *malta* showing *chlorosis* of leaves ($\times\frac{1}{2}$).
Note also wither-tip.

Sooty mould of *Citrus* leaves. ($\times\frac{3}{4}$).

Fig. 2

Fig. 3

Fig. 1

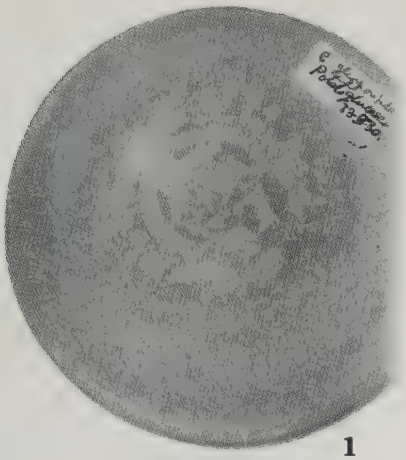
FIG. 1. Twig of *malta* showing wither-tip of all the branches and stem-ends of the fruits.
FIGS. 2 and 3 show stages in dropping of the fruits owing to stem-ends being attacked by *C. gloeosporioides*. ($\times\frac{1}{2}$).

FIG. 1. A *malta* tree badly infected by *C. gloeosporioides*. Most of the twigs and branches are dead. (×1/40.)

FIG. 3. A *malta* plant artificially inoculated. Note two of the branches are dead. (×1/16.)

FIG. 2 A *santra* tree almost dead owing to attack of *C. gloeosporioides*.

FIG. 4. Acervuli of *C. gloeosporioides* on *citrus* leaf produced by artificial



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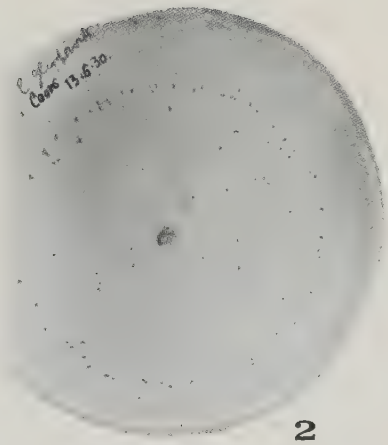

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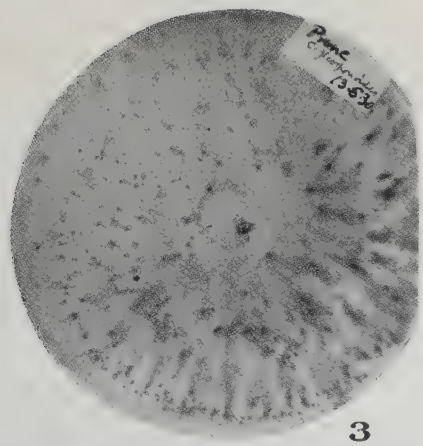

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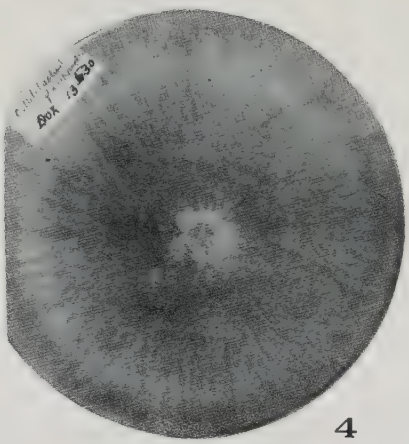

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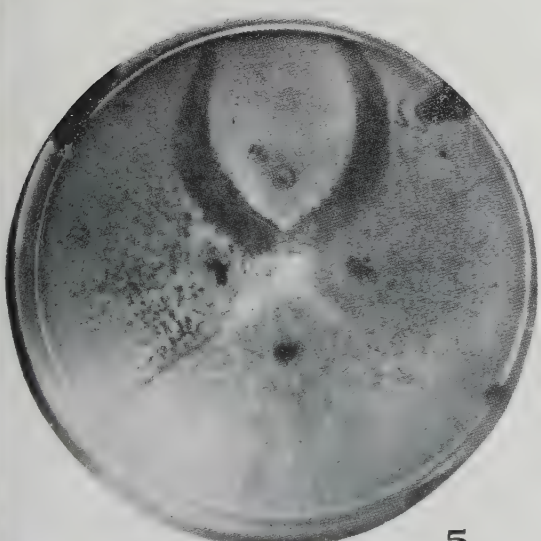

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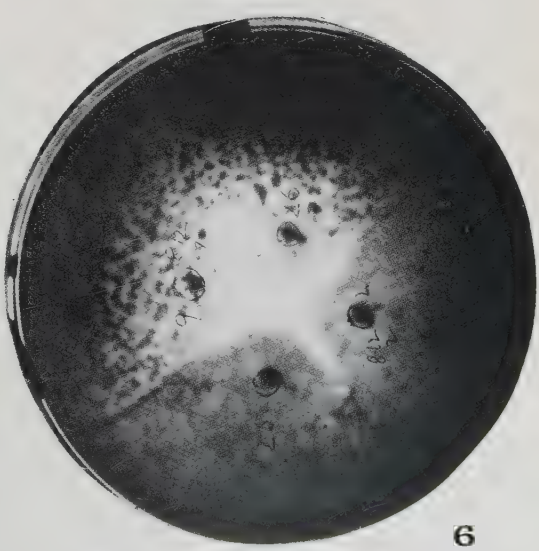
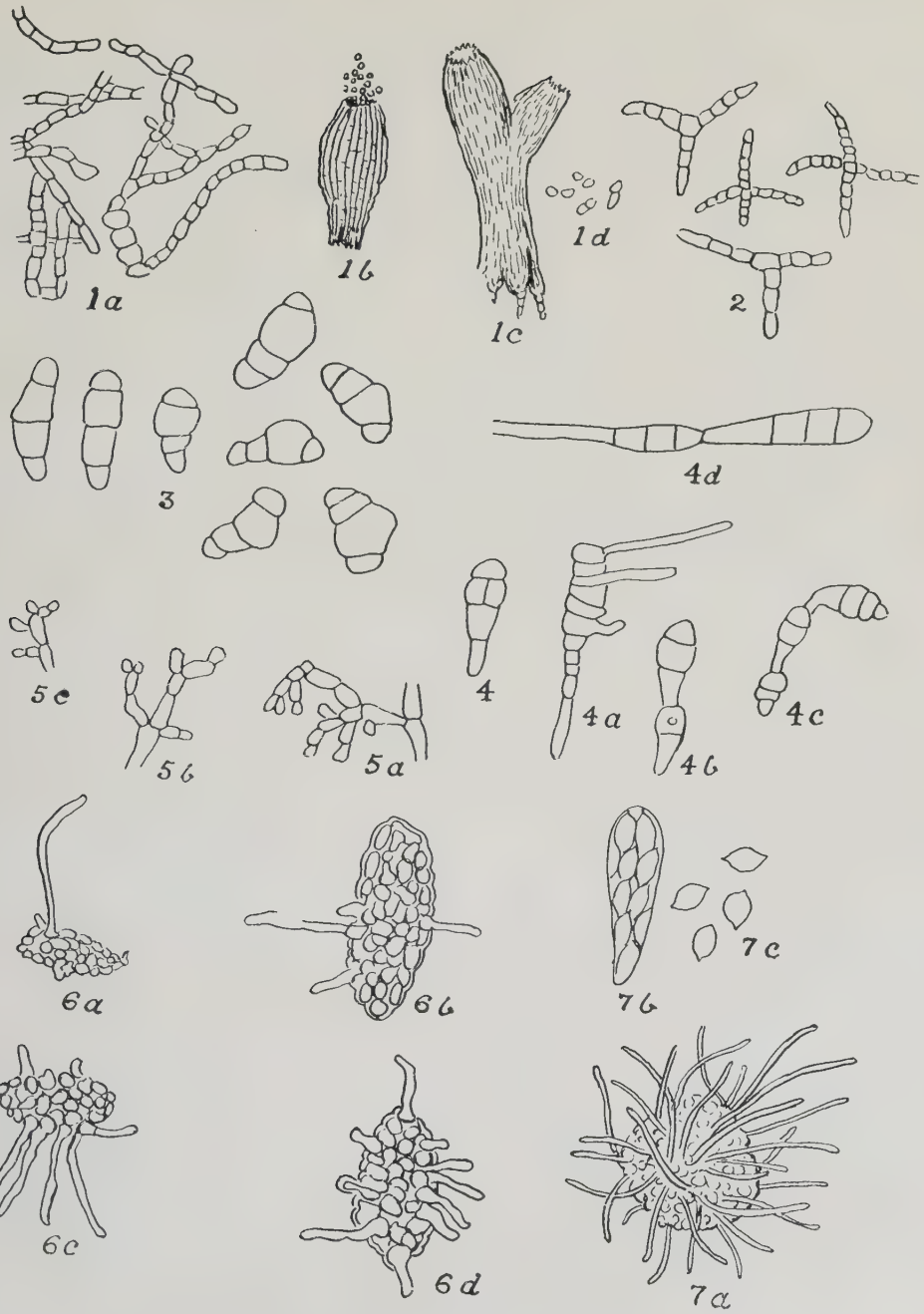


6

FIGS. 1, 2, 3, 4. Growth character of a strain of *C. glocosporiodies* on four different media. (×2/3.)

FIGS. 5, 6. Four different strains of *C. glocosporioides* inoculated at four different points of the Petri dish.

Note the space of aversion, white line of aversion and complete fusion in different cases. (×7/9).



SOOTY MOULD OF *CITRUS*.

FIGS. 1 and 2, *Capnodium citri*.

1. (*a*) Hyphae. Note some are segmented. (×480.) (*b*), (*c*), (*d*). Pyncidia and spores (×350).
2. Spores of *Triposporium* form (×350).

FIG. 3. Spores of *Acrothecium lunatum* (×610).

FIG. 4. Spores of *Alternaria citri* (×610).

In (*a*) hyphae being formed from each segment of the spores. In (*b*), (*c*) and (*d*) spores are being formed in chains.

FIG. 5. *Cladosporium herbarum* var. *citricola* (×750). Branching conidiophores bearing conidia.

FIG. 6. *Pleospora herbarum*, a-d (×450) germinating ascospores.

FIG. 7. *Chaetomium* sp. (*a*) Perithecia (×125), (*b*) ascus with ascospores (×650), (*c*) ascospores (×650).

FIG. 1. A twig of *malta* showing injury caused by hail-stone which later became infected by *C. gloeosporioides*.

FIG. 2. Anthracnose spots on leaves of *khatta*.

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A NEW VARIETY OF *OIDIOPSIS TAURICA*

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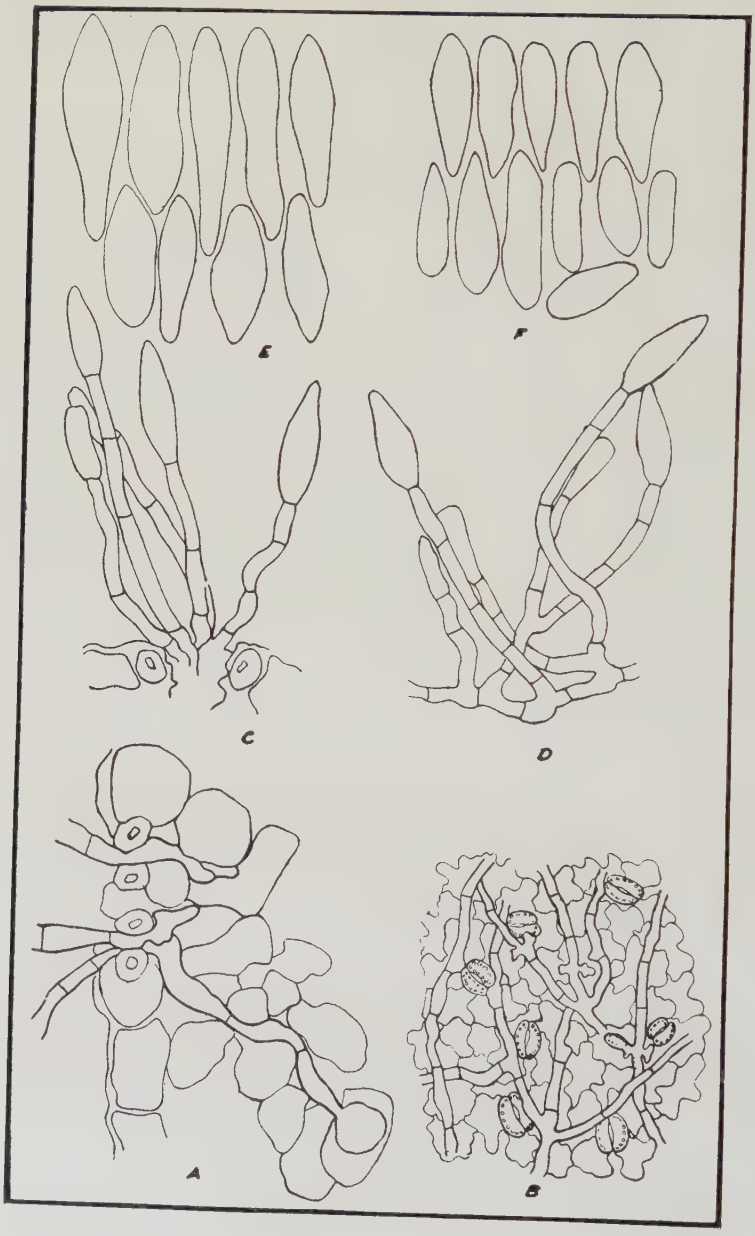
(With Plate XX and one text-figure)

In 1933 powdery mildew was observed on a single vine of *Dolichos lablab* L. in the grounds of Fergusson College in Poona. In general characteristics the fungus showed a close resemblance to *Oidiopsis taurica* (Lév.) Salm., which is a common parasite on a variety of plants in the Bombay Presidency. The conidia of the *Dolichos* fungus, however, were of unusually large size, and this character seemed to separate it from *Oidiopsis taurica*. The results of a study of this fungus are briefly described in the following pages.

PATHOLOGICAL EFFECTS OF THE FUNGUS ON THE HOST

In general, the pathological effects produced by the fungus on *Dolichos lablab* are very similar to those produced by *Oidiopsis taurica* on cluster bean, *Cyamopsis psoraloides* DC. The fungus forms on the upper surface of the leaves dull red spots, which are irregular in outline and are limited by leaf veins. On the under-surface of the leaves, white powdery patches of mildew are observed corresponding to the extent of the spots. The leaf dries up in the infected region, but in some cases the local discolouration associated with separate infected areas may become obliterated in the general invasion and the consequent drying up of the whole leaf. The affected plants are defoliated.



A. Transverse section of an affected leaf of *Dolichos lablab* showing endophytic mycelium ramifying in the intercellular spaces and in the sub-stomatal vesicles, and sending globular haustoria into the mesophyll cells. *B*. Portion of an affected leaf showing external mycelium, which possesses appressoria-like structures. *C*. Conidiophores emerging in fasicles through a stoma and bearing on their ends a single conidium. *D*. Branched conidiophores. External mycelium arising as lateral branches from the main axis of conidiophores. *E*. Conidia of *Oidiopsis taurica* var. *macrospora* showing range of shapes and sizes. *F*. Conidia of *Oidiopsis taurica* showing range of shapes and sizes.

Morphology of the fungus

The fungus at first is wholly endophytic. The mycelium ramifies in the inter-cellular spaces of the spongy and palisade parenchyma, and sends into the mesophyll cells small globular haustoria. The hyphae are septate and tortuous, and are abundant just below the epidermis and in the sub-stomatal vesicles. The epidermal cells are not invaded by the fungus (Plate XX, A).

From the internal mycelium, conidiophores arise and emerge singly or in fasicles through the stomatal apertures. The conidiophores are septate, sometimes simple, but more often branched, and are variable in length measuring up to 280 μ. They bear on their ends a single conidium. From the main axis of the conidiophores, lateral branches sometimes arise and spread indefinitely, forming the external mycelium (Plate XX, C and D). The hyphae of the external mycelium also emerge through the stomata, and in the later stages of the conidial phase, the mycelium is persistent, effused and often densely compacted, giving the appearance of white mealy growth. Sometimes short conidiophores may arise from the external mycelium (Plate XX, D). No haustoria are sent into the epidermal cells from the external mycelium, which nevertheless possesses structures similar to appressoria on the superficial mycelium of ectophytic powdery mildews (Plate XX, B). The external mycelium also forms feeding branches, which enter through the stomata to obtain nourishment from the inside of the leaf.

The conidia are extremely variable in shape and size. They are sub-cylindric, often more or less sub-acuminate towards the apex, or irregularly shaped with the middle part of the spore swollen or constricted, single-celled, and measure 47 to 111 μ × 10 to 34 μ (Plate XX, E). The conidia germinate by the formation of hyphae, which enter the host through the stomatal apertures and send haustoria into the mesophyll cells of the leaf. The germ-tube forms appressoria like the external mycelium.

The perithecial stage of the fungus was not found.

Cross-inoculation experiments

Curiously enough, the fungus on *Dolichos lablab* has been observed in nature on one or two vines only, and extensive search has failed to reveal it elsewhere. Evidence has been obtained that *Oidiopsis taurica* is highly specialised in its parasitism, and it is therefore difficult to determine whether the larger size of the conidia of the fungus on *Dolichos lablab* is due to the host influence. The sudden appearance of *Oidiopsis* on *Dolichos lablab* cannot be explained at present, and the work on host specialisation in *Oidiopsis taurica*, the results of which will be reported elsewhere, has also given no indications of the presence of any bridging

hosts. The fungus on *Dolichos lablab*, however, is capable of infecting this host under all conditions. The results of some cross-inoculation experiments are summarised in Table I.

TABLE I

Summary of results obtained by inoculating different hosts with conidia of Oidiopsis taurica *and of* O. taurica *var.* macrospora

Hosts inoculated	Conidia taken from			
	Dolichos lablab	*Cyamopsis psoraloides*	*Oxalis corniculata*	*Euphorbia geniculata*
Dolichos lablab . . .	*5/4	14/0	5/0	8/0
Cyamopsis psoraloides . .	7/0	13/11	5/0	9/0
Oxalis corniculata . . .	2/0	5/0	5/3	8/0
Euphorbia geniculata . .	2/0	9/0	3/0	9/7

* The numerator represents the number of plants inoculated, and the denominator the number of plants infected.

TAXONOMY

Salmon [1906] emended the genus *Oidiopsis* of Scalia to include those members of the Erysiphaceae which are chracterised by their completely endophytic habit during the early part of the conidial phase and produce branched conidiophores emerging singly or in bundles through the stomata. The fungus then known as *Erysiphe taurica* Lév. was transferred to this genus as *Oidiopsis taurica* (Lév.) Salmon. This species shows a wide variation in the size and shape of conidia and has been recorded on a large variety of host plants. The shape of the conidium, however, is often sufficiently constant and distinctive and does not vary as widely as the size.

As has been pointed out above, there is no evidence to show that the size and shape of the conidia of *Oidiopsis taurica* is affected to any appreciable extent by the host, although, as suggested by Salmon [1906], it seems probable that the variation shown by the conidia may be due to their position on the conidiophore.

The wide variation exhibited by the conidium is nevertheless sufficiently constant to make the fungus *O. taurica* easily recognisable. Salmon [1906] has, however, recognised a variety, *O. taurica* var. *lanuginosa* (Feckl.), on the basis of variation in the shape of the conidium.

Although the conidia of the fungus on *Dolichos lablab* fall within the range of normal form in shape usually encountered in *Oidiopsis taurica* (Plate XX, E and F), their size presents a feature most sharply distinguishing the *Dolichos* fungus. Fig. 1 and Table II will show that the conidia of *Oidiopsis* on *Dolichos lablab* are substantially longer than those of *O. taurica*, although there is not much difference in width. The conidia of the *Dolichos* fungus measure from 47 to 111 μ in length, whilst the range of length of the conidia of *O. taurica* as given by Salmon is from 32 to 82 μ.

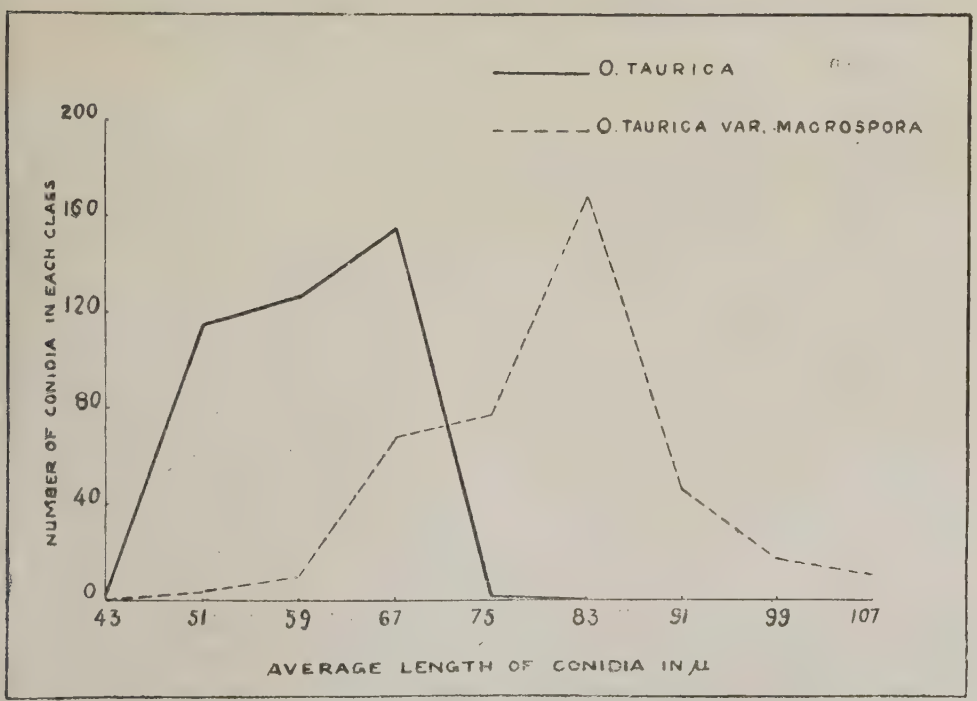


Fig. 1. Measurements of conidia of *Oidiopsis* on *Dolichos lablab* and those of *O. taurica*.

TABLE II

Comparative measurements of conidia of Oidiopsis taurica *on* Cyamopsis psoraloides *and of* O. taurica *var.* macrospora *on* Dolichos lablab

Classes in μ	Length		Classes in μ	Width	
	Number of conidia in 400			Number of conidia in 400	
	Dolichos lablab	*Cyamopsis psoraloides*		*Dolichos lablab*	*Cyamopsis psoraloides*
39 to 46·9 .	0	2	4 to 9·9	6	0
47 to 54·9 .	4	115	10 to 15·9	54	123
55 to 62·9 .	10	127	16 to 21·9	271	277
63 to 70·9 .	67	153	22 to 27·9	67	0
71 to 78·9 .	77	2	28 to 33·9	2	0
79 to 86·9 .	168	1	..	..	..
87 to 94·9 .	46	0	..	..	..
95 to 102·9 .	17	0	..	..	..
103 to 111 .	11	0	..	..	..

DIAGNOSIS

The *Dolichos* fungus differs from *Oidiopsis taurica* in the length of the conidium, a character which is constant and sufficiently distinctive to justify its separation as a variety. The name proposed for the new variety is intended to be descriptive of the large size of its conidia, thus emphasizing the important disgnostic character of the fungus.

Oidiopsis taurica var. *macrospora* var. *nov.*

External mycelium hypophyllous, usually persistent, effused, densely compacted, white. Internal mycelium ramifying in the inter-cellular spaces between the mesophyll cells and sending into these cells small globular haustoria.

Conidiophores septate, sometimes simple, but more often branched, emerging singly or in bundles through the stomata, measuring up to 280 μ, bearing single conidium on the end, also arising from the external mycelium, which possesses short appressoria-like structures.

Conidia extremely variable in shape and size, sub-cylindric, often more or less sub-acuminate towards the apex, or irregularly shaped with the middle part of the spore swollen or constricted, single-celled, measuring 47 to 111 μ × 10 to 34 μ, germinating by hyphae which enter the host through stomata and send haustoria into mesophyll cells of the leaf.

Attacking leaves of *Dolichos lablab* L., on the upper surface of which producing dull red spots and mealy white growth on the lower surface in areas corresponding to the spots, the leaf later drying up in the infected region. On one or two plants in the grounds of Fergusson College in Poona.

Type material deposited in the herbaria of the College of Agriculture, Poona, and of the Imperial Mycological Institute, London.

Summary

The fungus causing powdery mildew on *Dolichos lablab* has been described as a new variety of *Oidiopsis taurica*, from which it differs by the large size of its conidia. The name proposed for the *Dolichos* fungus is *Oidiopsis taurica* var. *macrospora*. The fungus produces dull red spots on the upper surface of the leaves of *Dolichos lablab*, the corresponding areas on the lower surface supporting white mealy fungus growth. This fungus has been observed on one or two plants only of *Dolichos lablab* in Poona.

The writers gratefully acknowledge the help received from Mr. S. F. Ashby, Mycologist, Imperial Mycological Institute, London.

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MICROSPOROGENESIS IN *CROTALARIA JUNCEA* LINN.

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(Received for publication on 24th April 1935)

(With Plates XXI and XXII)

The family Leguminosae contains nearly twelve thousand species, distributed all over the earth. Considering the large number of its species and their economic importance very little cytological and genetical work has as yet been carried out. Herbaceous plants of this family are commonly used as green manures and *Crotalaria juncea* Linn. is one of them. *Crotalaria juncea* is also one of the important fibre-yielding plants.

Microsporogenesis

The development of the pollen grains with reference to synizesis and chromosome conjugation in the family Leguminosae has received very little attention from cytologists. Latter [1926] worked on *Lathyrus odoratus* and suggested a telosynaptic method of chromosome conjugation. Her observations have, however, been criticised later by Maeda [1930]. Weintein [1926] worked out the cytology of *Phaseolus vulgaris*. He found the close approximation of two threads in late prophase. The segmentation of these threads gave rise to the bivalent chromosomes. Sethi [1930] working on *Cassia didymobotrya* found the spireme to be connected with the nucleolus and suggested a transference of material from the latter to the former. Reeves [1930] has worked out recently the microsporogenesis of *Medicago sativa*.

A comprehensive account of the chromosome number belonging to plants of this family has been recently given by Tischler [1931]. The chromosome number of *C. juncea* has been determined by many workers. Ramanujam, Parthaswarthy and Ramiah [1933] found eight to be the haploid number in four species of *Crotalaria*. Datta [1933], however, reports ten as the haploid number in *Crotalaria juncea*,

(For explanation see page 125)

Material and methods

Plants for this investigation were grown in the College experimental garden, and flower buds of various ages were fixed in the field on bright sunny days. Fixation between 12 noon and 4 P.M. gave best results. To facilitate the penetration of the fixing fluid an exhaust pump was used at the time of fixation, or the material was first dipped in acetic alcohol (1 : 2) for a short time before immersion in the fixatives. A number of fixing fluids were tried, but satisfactory results were obtained from flower buds fixed in Allen's modified Bouin's fluid. The material was dehydrated, cleared and embedded in paraffin in the usual way.

Microtome sections were cut of various thickness ranging from 8 to 16 μ according to the object of study. Sections were stained chiefly in Heidenhain's Iron-alum haematoxylin with or without a counter stain. Newton's Iodine Gentian violet stain was also used.

The resting nucleus

The last premeiotic division takes place rather rapidly, and in the same loculus preparatory stages for meiotic division and the resting pollen-mother cells can be observed. In the resting stage the pollen-mother cells are polygonal in outline and are closely compressed inside the anther loculus, there being no intercellular spaces. Each cell is bounded by a thin wall and is filled with finely granulated cytoplasm without vacuoles or dark staining bodies. The nucleus occupies more or less the central position.

The resting nucleus of the pollen-mother cell is nearly spherical in outline with a hardly distinguishable nuclear membrane. A large globular, deep staining nucleolus with a perinucleolar hyaline zone, occupies the central position. The hyaline zone is hardly traversed by peripheral granular strands (Plate XXI, fig. 1). The invariable occurrence of the perinucleolar hyaline zone, even in well-fixed material gives little support to McClung's [1929] contention that, it is due to the shrinkage of the nuclear contents away from the nucleolus because of the inadequate fixation of the karyolymph.

Heterotypic prophase

The activity of the nucleus starts with the appearance of a faintly staining granular reticulum at the periphery (Plate XXI, fig. 2). The reticulum gradually becomes coarser and the nucleolus also increases a little in size (Plate XXI, fig. 3).

Leptonema stage

The reticular condition is directly followed by the leptonema stage in which the network resolves into a fine continuous thread of granular appearance and is spread uniformly throughout the nuclear cavity (Plate XXI, fig. 4). The perinucleolar hyaline zone so far distinct is now lost sight of. No doubleness of the

thread nor any free end is observed at this stage. Some deep staining dots are always seen in association with the thread. These are slightly bigger than the net-knots which are formed by the crossing of several threads at a point, and cannot be considered as such. Their sudden appearance at this stage and disappearance abruptly in synizesis leads one to believe that their presence is due to some metabolic changes taking place in the protoplast at this stage.

Synizesis

The first indication of synizesis is seen by the withdrawal of the threads from the periphery towards the centre. The network gradually contracts uniformly from one side of the cell to the other, while the nucleolus retains its original position (Plate XXI, fig. 5). The entire network passes to one side of the nucleolus and remains as a contracted mass along the nuclear membrane without enclosing the nucleolus in its meshes (Plate XXI, fig. 6). It is not clear how the nucleolus escapes out of the reticulum and comes to lie at one side of the synizetic knot. It is too big to pass through its meshes and too viscous to be filtered through the net. Possbily the thread separates into two parts to allow the nucleolus to escape, but in the absence of corroborative evidence it is difficult to say anything definitely. The relative position of the nucleolus and the synizetic knot remains the same. The separation of the thread from the nucleolus is never complete and the knot is not a tight one, but is rather loose and the thread can be traced for a considerable distance before it becomes obscure (Plate XXI, fig. 7).

The later prophasic changes clearly show that chromosome pairing in this case is typically telosynaptic and so the actual significance of synizesis as defined by parasynaptists " the closer association of the homologues by their rearrangement " does not hold good here. But the synizetic knot in this case cannot be considered to be an artifact caused by the coagulation of the karyolymph as suggested by McClung [1929] or by its plasmolysis as Chodat [1929] suggests, since all the materials properly fixed with different fixatives present the same appearance of the synizetic knot. Sargant [1897] noticed the invariable presence of this stage in well-fixed material and even in living material. Though synizesis seems to be an essential stage through which the spireme passes in meiosis, its actual significance in some cases is open to question, and its absence again in some species of Oenothera is peculiar and challenges the essentiality of such a stage.

Open spireme

The contracted spireme opens out gradually by throwing irregular loops towards the distal end (Plate XXI, fig. 8). The thread is rather uniform in appearance, but very slender and does not stain deeply. Following this stage the pollen-mother cells gradually lose their polygonal shape and round off. When the synizetic knot is fully unravelled it fills up the entire nuclear cavity. A

typical hollow spireme results and the thread is disposed inside the nucleus in large irregular coils (Plate XXI, fig. 9). No free end of the thread (except the cut ends), nor their dual nature is clear at this stage. Whenever free ends are seen they are invariably at focal extremes suggesting them to be cut ends of a continuous spireme, the remaining segments in the consecutive sections justifying this contention.

Pachynema

The thread thickens up by contraction and the loops become more conspicuous (Plate XXI, fig. 10). The loops are so wide that it is difficult to count their number accurately, but careful observation shows that they do not exceed the haploid number of chromosomes of the plant. The continuity of the thread is very clear, no free ends being visible, and it is uniformly thickened all through. The nuclear cavity is very much expanded and the mother cells round off completely and become mostly independent of one another.

Second contraction or brochonema

The spireme gradually condenses and the number of loops become distinct (Plate XXI, figs. 11 and 12). As far as can be made out the loops are continuous with one another. The number of persistent loops is the same as the haploid number of chromosomes, suggesting as if each loop is composed of a pair of chromosomes joined end to end. The folds of the thread forming the loops lie mostly about the nucleolus and when not so, the central knot is not too far away from it. Further condensation of the thread produces a characteristic orientation of the loops. It appears as if the loops are radiating from the centre where the nucleolus is situated.

The loops twist in various ways turning through different angles round their own axis. The nodes or chiasma so formed by the twisting of the loops suggest at once as if they bring about an interchange of genes between two components of a pair of homologues, which is otherwise insufficient by their union at one place at the end. This interpretation gets more support due to less number of nodal regions in the smaller pair, where in comparison with their smaller size only one point of union is sufficient for the interchange. The number of chiasma so far obtained is a constant character for a particular pair.

Early diakinesis

Further contraction of the spireme leads to the segmentation of the loops from the knot but the actual mechanism of this process is not clearly understood (Plate XXI, fig. 16). Some of the loops which lie away from the knot are seen still to be connected with it by a fine strand of dark staining material (Plate XXI, fig. 15), and if this strand be taken as a part of the true chromosome-forming

substance which is drawn into its total composition afterwards, then the mechanism of breaking can be explained as due to the contraction of the chromatin of each loop separately from the knot, and the ground matrix of the chromosomes is left over forming the connecting link. This link gives way later being less tenacious and thus the separation of the loops is brought about. This view gets support from the evidence obtained in the late-diakinetic stages where chromatin of each chromosome contracts leaving a hyaline connection at the tip of the previous loop, and therefore it can be suggested that the segmentation of the spireme is due to the condensation of the chromatin of the homologous pair whereas the separation of the homologues is by the contraction of the individual chromosomes. If on the other hand the thin strand be considered as not taking any essential part in the formation of the chromosomes but later dissolves freely in the nuclear cavity, then the mechanism of segmentation of the loops can be considered from a different point of view, *viz.*, the dissolution of the chromatin of each loop near the knot. This transference of chromatin material from the chromosomes to the karyolymph is evidenced by the fact that the nuclear cavity now contains some deep staining material. But the simultaneous occurrence of similar deep staining bodies in the cytoplasm goes against this view (Plate XXI, figs. 14-18).

Diakinesis

When the segmentation of the loops is complete, the separate pairs lie scattered in the nuclear cavity. The two arms of each loop separate out and they appear to be very much thickened. The two free ends of each pair, so far quite apart, now come to lie closer together, causing them to appear as rings or figure 8. Besides the forms 0 and 8, they often assume the forms of V and Y (Plate XXI, figs. 17-19). The figure 8 form may be due to the twisting of the rings or due to the unfolding of the primary twists of the loops. The different pairs can be classified according to their lengths even at this stage of condensation, and this gives the graphic formula $2S + 5M + 1L$

Further condensation of the individual chromosomes of a homologous pair throws much light on their mode of conjugation. The chromatin of each pair contracts with a definite sequence from the point of union of the two homologues leaving a hyaline zone at the tip of the loop suggesting thereby that the paternal and the maternal chromosomes are joined end to end and their point of union is at the tip of the brochonema loops.

Plate XXI, fig. 18 shows some of the chromosomes with hyaline zone at the point where they are attached to their homologues. This shows that the chromosomes are composed of two substances : the basophilic chromatin and another hyaline ground substance which most probably forms the dispersion medium.

The two arms of the bivalents thus break apart at their point of union and the two components either lie in a ring or side by side in close approximation (Plate XXI, fig. 19).

(For explanation see page 125)

The deeply stained nuclear and cytoplasmic bodies, which were at first noted in the early-diakinetic stage and were present so far, now disappear from all parts of the cell.

Spindle formation

The nuclear membrane disappears abruptly before any spindle fibres are noted either as polar caps or inside the nuclear cavity. It is clear that the nuclear membrane becomes gradually thinner in the late-diakinetic stage. With the disappearance of nuclear membrane the scattered chromosomes clump together in the centre surrounded by a hyaline zone. This hyaline area is somewhat coarser and striated, suggesting intra-nuclear origin of spindle fibres (Plate XXII, fig. 20).

The components of a homologous pair establish more intimate contact and no clear space is evidenced in between them, but they still retain their rod-shaped structure. No multipolar spindle is observed. The spindle when first noted shows the fibres arranged in straight rows (Plate XXII, fig. 21), but when its bipolar nature is completely established, some of the fibres appear to be considerably thicker, specially towards the poles. They are fewer in number and their actual attachment to the chromosomes is not clear. The spindle formation is so rapid that stages from diakinesis to heterotypic metaphase are observed in the same loculus.

Heterotypic metaphase

The gemini which clumped in the centre of the nucleus separate out and their bivalent nature is apparent even in their maximum stage of condensation. Polar view of an equatorial plate shows eight such bivalents indicating thereby that the haploid number is eight (Plate XXII, fig. 23). The spindle now shows distinct polarity and the converging fibres meet at two sharp points. The bivalents are arranged lengthwise along the equatorial region of the spindle with each component towards each pole, the constriction between them being in the centre (Plate XXII, fig. 22). The nuclear zone formerly unoccupied by the cytoplasm and so long distinct is now encroached upon by the cytoplasm. But the cytoplasm surrounding the spindle remains less dense than the peripheral zone of the cell.

Heterotypic anaphase and telophase

The constriction of the bivalents in the equatorial zone of the spindle now becomes more prominent and well-marked split appears (Plate XXII, fig. 24). The chromosomes now disjoin and the univalents, spherical in shape, move towards the poles. Their movement to the poles is not very regular, one chromosome belonging to each haploid set moves earlier (Plate XXII, figs. 25 and 26)

and reaches the pole before the rest (Plate XXII, fig. 27). In some cases one or two chromosomes are seen to lag behind the rest. When the chromosomes reach the poles, they clump together and their individuality cannot be made out (Plate XXII, fig. 28).

Before the disappearance of the spindle fibres, the chromosomes get separated from one another and look distinct in a telophasic plate (Plate XXII, figs. 29 and 30). The homotypic split of the univalent chromosomes which could not be made out even in the late anaphase now appears all on a sudden and in some the split is quite apparent.

In late telophase the spindle fibres completely disappear but a fine cytoplasmic zone is left round about the polar region. The split becomes complete and the dual nature of each univalent chromosome is evident. Most of the univalents have the two split-halves lying side by side or in the form of a cross (Plate XXII, fig. 31). In the latter case this is probably due to the separation of the two halves from one another at two ends, being connected only at the central region, so that one half swings across the other approximately at right angles to it. Previous to this, when the split was not complete but the dual nature was prominent, the constriction appeared to be transverse, due probably to the extreme condensation of the chromosomes. This apparent transverse constriction cannot be otherwise interpreted, because of the spherical nature of the univalents at this stage. It is a well-known fact that the plane of division of a sphere is always transverse. But from the relative sizes and parallel association of the components of the univalents, it is clear that the split is a longitudinal one and not transverse.

Interkinesis

A clear zone appears surrounding the chromosome region; most probably this is due to the deposition of the nuclear sap; and the nuclear membrane appears simultaneously with the appearance of the nucleolus. The size of the nucleolus is very small at this stage. The chromosomes elongate very much, sending out chromatin strands which intertwine and anastomose freely between them (Plate XXII, fig. 32). The chromosomes do not resolve into complete threads and their number can be easily counted, though they do not retain their original form and their double nature is indistinct.

Homotypic division

Before the formation of the homotypic spindles the nuclear membrane becomes thinner. Very faint fibrillar structures appear in between the chromosomes, suggesting intra-nuclear origin of the homotypic spindles (Plate XXII, fig. 34). The nucleolus is not clearly seen at this stage, due to clumping of the chromosomes. Later the membrane disappears completely and the chromosomes

become distinct from one another. No trace of the nucleolus is observed. The homotypic spindles are always bipolar but their space relationship is very variable, they may lie parallel or at right angles to each other (Plate XXII, figs. 35 and 36).

The univalent chromosomes in a homotypic equatorial plate appear mostly as crosses and a few as V or Y (Plate XXII, fig. 36). The appearance presented at this stage is very similar to that observed in the late telophase before the organisation of the inter-kinetic nucleus (Plate XXII, fig. 31).

The chromosome halves, already split, move apart and reach the poles very quickly. The chromosomes now appear spherical without any split or constriction and all of them do not move to the pole with the same velocity, some moving earlier and some lagging behind (Plate XXII, fig. 37).

Spindle fibres do not disappear until the individual chromosomes in the telophase get separated from their aggregated condition formed in the early telophase (Plate XXII, fig. 38). A polar plate of this stage shows the appearance of constriction in the chromosomes (Plate XXII, fig. 39). This split is a preparation for the first division in the microspore.

A clear zone appears in the polar region due to the deposition of the nuclear sap, and the nuclear membrane appears simultaneously with the appearance of the nucleolus. No spindle fibres nor any trace of them are observed at this stage. The chromosomes resolve into chromatin threads but not completely until cytokinesis begins. Thus the tetrahedral arrangement of the granddaughter nuclei is established and an examination of a large number of such stages shows that this is not always due to the decussate arrangement of the spindle fibres.

The nucleus gradually increases in size and the chromosomes anastomose with one another sending chromatin strands in all directions. They lose their deep staining property and the appearance of such anastomosis is an indication of the formation of a resting reticulum.

Cytokinesis takes place by the process of furrowing. The microspores are liberated by the dissolution of the thickened mucilaginous layer. The mature pollen grains are somewhat elongated and uni-nucleate. The pollen grains of the linear and globose anthers are similar in shape and size.

Summary

1. The resting nucleus of the pollen-mother cell shows a granular zone along the peripheral region and a hyaline perinucleolar area.

2. The spireme remains granular in the leptonema and synizetic stages.

3. The spireme is continuous all through. No longitudinal split or parallelism of threads has been observed.

4. Brochonema loops are equal to the haploid number of chromosomes.

5. Diakinesis is normal and the bivalents exhibit the following forms :— V, O, X and Y. In the late diakinesis the components of each homologous pair contract leaving a hyaline connection at their point of union.

6. Some deeply staining bodies occur in early-diakinetic stage simultaneously both inside and outside the nucleous. These disappear in the late diakinesis.

7. Absence of parallelism of the leptonema and pachynema threads, the typical brochonema stage with the loops equal to the haploid number of chromosomes and the separation of each loop into two univalents suggest that the method of chromosome pairing is telosynaptic, where the paternal and the maternal chromosomes are joined end to end in the spireme.

8. The heterotypic metaphase is normal. In anaphase two chromosomes are seen to move faster than the rest, one to each pole.

9. Polar view of an equatorial plate at heterotypic metaphase shows eight tivalent chromosomes.

10. Homotypic split is first noticed in heterotypic telophase and the split is so complete that the chromosomes appear in the forms of X, V or Y.

11. There is a very short interphase between heterotypic and homotypic divisions.

12. The origin of both heterotypic and homotypic spindles appear to be intra-nuclear.

13. Homotypic division is normal. The space relationship of the spindles is variable.

14. Cytokinesis takes place by furrowing.

15. Mature pollen grains are uni-nucleate and lie scattered in the loculus.

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Explanation of Plates XXI and XXII

The figures were drawn with the aid of a Camera lucida at table level. The magnification of each of the figures is 2,400.

Plate XXI

Fig. 1. A resting pollen-mother cell with a granular peripheral zone and a hyaline perinucleolar zone.
Fig. 2. Very early prophase ; early indication of thread formation is evident.
Fig. 3. Advanced reticulum.
Fig. 4. Leptonema. Thread single and univalent.
Fig. 5. Early synizesis. The reticulum contracts from the nuclear membrane.
Fig. 6. The massing of the reticulum to one side of the nuclear cavity.
Fig. 7. Typical synizesis.
Fig. 8. Loosening of the spireme after synizesis. No indication of the granular appearance of the thread remains.
Fig. 9. Hollow spireme.
Fig. 10. Pachynema.
Figs. 11 & 12. Second contraction. Eight radiating loops are seen.
Fig. 13. The eight persistent loops of the previous figure are drawn separately, showing the position of each with regard to the central nucleolus.
Fig. 14. Later stage of second contraction.
Fig. 15. Segmentation of the loops to form the bivalent chromosomes.
Fig. 16. Early diakinesis.
Fig. 17. Diakinesis.
Fig. 18. Contraction of the individual chromosomes of the gemini resulting in the partial separation of the univalents.
Fig. 19. Side-by-side association of the homologues after their separation (composite drawing from two sections).

Plate XXII

Fig. 20. Alignment of the chromosomes in the equatorial plate prior to the formation of the spindle. The nuclear membrane and the nucleolus have completely disappeared.
Fig. 21. First appearance of the bipolar spindle.
Fig. 22. Heterotypic metaphase.
Fig. 23. Polar view of an equatorial plate showing " eight " bivalent chromosomes.
Fig. 24. Early anaphase. Note separation of the univalents.
Figs. 25 & 26. Anaphase. Note early migration of a single chromosome to each pole.
Fig. 27. Late anaphase. Two chromosomes stand out separate at the poles. They have reached earlier.

Fig. 28. Telophase.
Fig. 29. Separation of the individual chromosomes at telophase.
Fig. 30. Polar view of a telophasic plate. Appearance of the homotypic split in the chromosomes just apparent in this stage.
Fig. 31. Late telophase. Components of univalents appear mostly as crosses.
Fig. 32. Interkinesis. Note the drawn out condition of the chromosomes.
Fig. 33. A late stage showing eight split chromosomes.
Fig. 34. Intra-nuclear origin of the homotypic spindle.
Fig. 35. Homotypic metaphase.
Fig. 36. Polar view of homotypic metaphase. Note the split condition of the chromosomes.
Fig. 37. Homotypic anaphase.
Fig. 38. Homotypic telophase.
Fig. 39. Chromosomes in telophase showing their constricted nature.

RESEARCH NOTE

EFFECTS OF BROWN RUST ATTACK ON WHEAT

BY

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(Received for publication on 31st October 1935)

Johnston and Miller (*J. Agric. Res.* 1934 : **49** : 955-81) studied the effect of brown rust infection on the growth, yield and water economy of Warden and Pusa 4 wheats. Physiologic form 9 of brown rust (or leaf rust or orange rust as it is alternatively called) was used in these investigations. Pusa 4 is susceptible to this physiologic form while Warden is highly resistant to it.

Seeds were sown in sealed one-gallon stone jars. For each variety there were five groups of six jars containing six plants each. One group of each variety was maintained to maturity as a rust-free control while the remaining four groups of each variety received their initial inoculation with brown rust at the seedling, jointing, booting* and flowering stages, respectively. After this each inoculated group was re-inoculated sufficiently often to ensure abundant infection.

The data show that brown rust infection greatly reduced the yield of grain in the susceptible variety. The loss ranged from 42·4 per cent when infection took place at the flowering stage to as much as 93·8 per cent when infection commenced at the seedling stage. In the case of the resistant variety also there was some loss in grain yield. The earlier the infection, the greater was the loss. The yield reduction was principally brought about by the production of fewer kernels per head and, to a lesser extent, by a reduction in the weight of the individual kernels. The average yield of total dry matter and straw was also considerably reduced in the case of the susceptible variety, though not to the extent of the grain yield. In the case of the resistant variety, the reduction was small at all stages and probably within the experimental error.

A striking effect of heavy rust infection in the case of the susceptible variety was the marked deterioration of the roots as indicated by discolouration, loss of weight and decrease in the number of fibrous roots. The groups that were infected late showed about the same amount of damage as those infected early in the season. In the case of the resistant variety, however, no deterioration was observed.

* Booting = the stage just before the ears emerge when they are still enclosed by the sheath.

Brown rust infection also caused a reduction in the number of heads and height of plant, prolonged the time required to reach full heading and maturity, and increased the water requirements of plants, in the case of the susceptible variety. Similar effects were noted in the resistant variety too but on a much smaller scale.

The interesting and important results of Johnston and Miller summarised above lead one to a consideration of the position with regard to brown rust of wheat in India. Whereas in the great wheat-growing regions of Canada and the United States of America, black rust appears to be the most important rust, in India brown rust and yellow rust are equally important. In fact in Bihar and the United Provinces where the growing period for wheat is comparatively short (November-March), experience indicates that black rust generally arrives too late in the season to be able to do much damage, and it is the brown and yellow rusts which are mainly responsible for the reduction in yield.

As in the case of the other two rusts, physiologic forms of brown rust are known to exist, that is to say, strains of the parasite exist which are morphologically indistinguishable from each other but which differ in their ability to infect different varieties of wheat. Over fifty physiologic forms of brown rust are known and the number is increasing as more work in various parts of the world is done. In India two physiologic forms—Form 10 and another—have been distinguished so far [Mehta, 1933]*. Of the Indian wheats tested, none is resistant to the first form but the newer Pusa varieties, Pusa 114, Pusa 120, Pusa 125, and Pusa 165, are highly resistant to the second form.† Physiologic form 9 with which Johnston and Miller worked has not so far been recorded in India.

The breeding of rust-resistant wheats for India has recently been taken up at Pusa and at a sub-station at Simla, in collaboration with Prof. K. C. Mehta of Agra and with the help of a grant from the Imperial Council of Agricultural Research. Until Prof. Mehta took up work on this subject, it was not known as to what physiologic forms of the three rusts existed in India and although some striking successes were obtained, notably at Pusa, previous breeding for rust resistance may be said to have been carried on in the dark. Now however a definite scientific procedure is possible. A large number of wheat varieties is being tested for resistance to the Indian physiologic forms of rust. As stated above, none of the Indian varieties yet tested is highly resistant to both the physiologic forms of brown rust occurring in India. Fortunately, however, a few foreign varieties are known to be resistant and these will be crossed with the best of the Indian wheats with the object of securing a wheat combining resistance to rust with other economically desirable qualities. It is hoped to obtain resistance not only to brown rust but also to the black and yellow rusts.

* K. C. Mehta, 1933. *Ind. J. Agric. Sci.* **3** : 939-62.

† K. C. Mehta—Unpublished data.

NOTICE

BIBLIOGRAPHY OF SOIL SCIENCE, FERTILIZERS AND GENERAL AGRONOMY

The Imperial Bureau of Soil Science has recently issued a complete " Bibliography of Soil Science, Fertilizers and General Agronomy " covering the whole scientific literature of the world on soil and allied sciences during 1931-1934. This extremely comprehensive bibliography gives the reference to practically every paper published during that period on the different branches of soil science, both pure and applied, on the use of fertilizers, on the cultivation of all the chief economic crops (over 140, including forests, are listed in the index) and on plant diseases in relation to soils. The references are arranged by subjects according to the Universal Decimal Classification, which is adequately explained in the Preface. The volume also contains an index to the Decimal Classification, an alphabetical cross-index to every subject on which the papers listed have been written, an author index containing over 4,000 names, and a list of the abbreviations used, and the full titles and places of issue (where known) of 800 journals, etc., from which the references in the Bibliography have been taken.

The Bibliography has been compiled from the references which appeared in the first 41 issues of the Bureau's monthly lists of " Publications relating to Soils and Fertilizers ". An unusual feature is that the literature of almost every country has been covered equally well, thanks to the exceptional facilities for procuring foreign periodicals which the Soil Bureau enjoys. Considering its scope and the amount of information it contains, the book has been made remarkably compact, without detriment to its readability. It should be a most valuable reference book to every agricultural scientist whose work is in any way connected with the soil. The Bureau intends to issue further similar bibliographies at intervals of 3-4 years, and in this way gradually to build up a complete reference library to the whole literature of the soil over an extended period of time.

Crown octavo, pp. 504. Bound in cloth, with gold lettering, price 25/- net, post free from the Imperial Bureau of Soil Science, Harpenden, England.

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The Bearing of the Physiology of Parasitic Nematodes on their Treatment and Control (post free) . . . . . 3*s.*

VI. Obtainable from the Imperial Bureau of Animal Genetics, Institute of Animal Genetics, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh

Journal

Price

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VII. Obtainable from the Imperial Bureau of Animal Health, Veterinary Research Laboratory, New Haw, Weybridge, Surrey

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Indexing Publication

Index Veterinarius : Four issues a year. Annual subscription (postage paid) . . . . . 80*s.*

VIII. Obtainable from the Imperial Bureau of Animal Nutrition, Rowe Research Institute, Bucksburn, Aberdeen

Journal

Nutrition Abstracts and Reviews :—Issued under the direction of the Imperial Agricultural Bureau Council, the Medical Research Council and the Reid Library. Subscription per Volume of 4 numbers . . . . . 21*s.*

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Annual subscription (payable in advance) Vol. XXII (1934) . . 24*s.* 12*s.*

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Index (post-free) Single part . . . . . 2*s.*

Title page and index . . . . . 3*s.*

Occasional Publication

Report on the Third Mycological Conference, 1934.

APPENDIX

IMPERIAL COUNCIL OF AGRICULTURAL RESEARCH

INSTRUCTIONS TO AUTHORS OF PUBLICATIONS OF THE IMPERIAL COUNCIL OF AGRICULTURAL RESEARCH*

1. All manuscripts should be clean, clear and carefully revised. Only one side of the paper should be used, and as far as practicable the original type-written copy and not a carbon copy should be sent. Capitals should be sparingly used, and all the necessary punctuation should be done in the MS. and not left for introduction in proofs.

2. The title of a paper should not be lengthy.

3. It is desirable that the MS. should have suitable heads and sub-heads. In numbering the principal divisions of a paper roman numerals should be used. The use of arabic figures and (*a*), (*b*), (*c*), etc., is generally reserved for numbering the subdivisions coming under each head.

4. Articles submitted for publication either in the *Indian Journal of Agricultural Science* or in the *Indian Journal of Veterinary Science and Animal Husbandry* should be accompanied by abstracts for publication in *Agriculture and Live-stock in India*. Abstracts should be concise, but should be long enough to explain the matter dealt with ; ordinarily no abstract should exceed 200 words.

5. When a word or line is intended to be printed in *italics* it should be underlined with a single line, in SM. CAP. with two lines, in CAPITALS with three lines, and when in **Antique** (heavy type) with a wavy line (~~~~~~~~~~).

6. In descriptive matter, numbers under 100 and all numbers occurring at the beginning of a sentence should be in words.

7. Local names for crops, technical operations, etc., should be defined where they first occur in the text, *e.g.*, *rabi* (spring crop). The use of local weights and measures should be avoided as far as possible. Vernacular names, such as *jowar*, *bajri*, should be in italics without a capital letter, and each such name where it first occurs should be followed by its scientific equivalent in brackets, *e.g.*, *jowar* (*Andropogon Sorghum*). It is usual to write the initial letters of varietal names in capitals, *e.g.*, Striped Mauritius, Dharwar-American cotton and Broach cotton.

8. Botanical and zoological names are printed in italics and sh ould be underlined in the MS., *e.g.*, *Triticum vulgare* L. ; *Diplodia Corchori* Syd.; *Pyrilla aberrans* Kirby. The International Rules of Botanical Nomenclature and the

* Spare copies of these Instructions can be had on application to the Secretary, Imperial Council of Agricultural Research (Publication Section), New Delhi.

International Rules of Zoological Nomenclature should be followed. The names of chemical substances should not be written with a capital letter ; they are printed in roman type (*e.g.*, calcium carbonate, prussic acid).

9. The following and similar abbreviations may be used freely :—*viz.*, *e.g.*, *i.e.*, mm. (millimetre), cm. (centimetre), grm. (gramme), mg. (milligramme), c.c. (cubic centimetre), sp. gr. (specific gravity), lb. (pound), cwt. (hundredweight), in. (inch), ft. (foot), oz. (ounce), md. (maund), sr. (seer), ch. (chattack). Other abbreviations should be used sparingly, if at all.

10. References to plates should be given within brackets, without prefixing the word " see " or " cf.", in the MS. itself, and should not be left over for introduction in proofs. For example, " The parasite (Pl. X, fig. 4) was present late in 1906 ".

11. The word " Table " is preferable to " Statement ", and tables should be numbered consecutively in roman figures. Each table should have an explanation as a sub-head. It is more convenient for reference if tables can be printed horizontally ; for this purpose they should not exceed in width the printing measure of the page (5 in.). *Example—*

TABLE IV

Results of water-saving experiments on wheat (Pusa 12) at Gungapur, Haripur and Sargodha, 1916-17

Station	No. of irrigations including the preliminary watering	Yield per acre in maunds and seers		Average yield per acre	
		Grain	Straw	Grain	Straw
		Mds. Srs.	Mds. Srs.	Mds. Srs.	Mds. Srs.
Gungapur . . .	One	12 19½	20 10	9 34	21 17
Haripur . . .	,,	8 31	19 14		
Sargodha . . .	,,	8 12½	25 27½		

12. References to literature, arranged alphabetically according to authors' names, should be placed at the end of the article, the various references to each author being arranged chronologically. Each reference should contain the name of the author (with initials), the year of publication, the abbreviated title of the publication, volume and page. In the text the reference should be indicated by

the author's name followed by the year of publication enclosed in brackets ; when the author's name occurs in the text, the year of publication only need be given in brackets. If reference is made to several articles published by one author in a single year, these should be numbered in sequence and the number quoted after the year both in the text and in the collected references. This system of referencing is the same as is used in the *Biochemical Journal* with slight modification and will be clear from the following illustration :—

The work of Osborne and Mendel [1919, 1, 2] and Steenbock and Boutwell [1919] had indicated an association of the fat-soluble vitamin with the green parts of plants. This view was examined by Coward and Drummond [1921] who reported that vitamin A was not synthesised by etiolated shoots but that green leaves were active in its formation. Another worker [Wilson, 1922], on the other hand, found that etiolated shoots if given in sufficient quantity could supply the fat-soluble vitamin and that this factor was therefore formed in the absence of light.

REFERENCES

Coward, K. H. and Drummond, J. C. (1921). *Biochem. J.* **15**, 530.
Osborne, T. B. and Mendel, L. B. (1919, 1). *J. Biol. Chem.* **37**, 187.
——————————————(1919, 2). *J. Biol. Chem.* **41**, 549.
Steenbock, H. and Boutwell, R. (1919). *J. Biol. Chem.* **41**, 149.
Wilson, J. (1922). *J. Biol. Chem.* **51**, 455.

Abbreviations, as far as possible, should follow the system adopted in " A World List of Scientific Periodicals " published by the Oxford University Press.

13. Papers should be complete when submitted for publication. As alterations and additions at the proof stage cause both additional expense and delay, they should be resorted to as little as possible. In making corrections in proofs the recognized symbols which will be found in the " Standard Dictionary " should be used. Second (page) proofs will be submitted to authors who should return them promptly.

Illustrations.

14. As the *format* of the journals has been standardized, the size adopted being crown quarto (about $7\frac{1}{8}'' \times 9\frac{5}{8}''$ cut), no text-figure, when printed, should exceed $4\frac{1}{2} \times 5$ inches. Figures for plates should be so planned as to fill a crown quarto plate—the maximum space available for figures being $5\frac{3}{4} \times 8$ inches exclusive of that for letterpress printing.

15. Photos or drawings for illustration should accompany the manuscript and each should bear on the reverse side the name of the paper to which it relates together with the title or legend, figure or plate number, and the size to be reproduced. When giving instructions for reduction linear measurements are understood ; thus, " half-size " means reduce to half the length and breadth, not half

the area. A photograph should not be rolled up, nor pinned, and should always be packed flat. A complete list of plates and figures should always accompany the paper.

16. Line drawings should be made with clear black lines on smooth white paper, preferably Bristol board. Rough paper should be avoided. Care should be taken that all the lines are drawn firmly ; scratchy or grey lines, produced by the ink being thinned down, are not permissible. Drawings should be larger than the required size. All lettering should be neatly and clearly put in, care being taken to make all lettering sufficiently large to stand reduction.

17. For half-tone work, copy should be made on glossy silver paper and of the same size or larger than the size required.

18. For three-colour work, copy may be oil-painting, water-colour, coloured photograph or coloured transparency, and larger than the size required. In preparing copy, one should use only the primary colours, in any combination, as only inks of primary colours are used in printing. Originals can be enlarged, if necessary, but this should be avoided if possible.

19. For detailed instructions regarding preparation of illustrations, it would be of advantage to refer to Mr. C. M. Hutchinson's article on " Photographic Illustrations " in the *Agricultural Journal of India*, Vol. XI, Pt. 3, July 1916, and Mr. A. W. Slater's paper on " The Preparation and Reproduction of Scientific Illustrations " in the *Proceedings of the Third Entomological Meeting*, 1919, which has been reprinted as *Bulletin No. 114 of the Agricultural Research Institute, Pusa.*

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